HYDROTHERMAL EXPERIMENTS ON THE THERMAL STABILITY OF AMINO SUBSTANCES IN SEDIMENTS

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ABSTRACT

Aspartic acid, threonine, serine and other thermally unstable amino acids have been found in fine-grained clastic sediments of advanced geologic age. The presence of these compounds in ancient sediments conflicts with experimental data determined for their simple thermal decomposition.

Recent and Late Miocene sediments and their humic acid extracts, known to contain essentially complete suites of amino acids, were heated with H_2O in a bomb at temperatures up to 500°C in order to compare the thermal decomposition characteristics of the sedimentary amino compounds.

Most of the amino acids found in protein hydrolyzates are obtained from the Miocene rock in amounts 10 to 100 times less than from the Recent sediment. The two unheated humic acids are rather similar despite their great age difference. The Miocene rock appears uncontaminated by Recent carbon.

Yields of amino acids generally decline in the heated Recent sediment. Some amino compounds apparently increase with heating time in the Miocene rock.

Relative thermal stabilities of the amino acids in sediments are generally similar to those determined using pure aqueous solutions. The relative thermal stabilities of glutamic acid, glycine, and phenylalanine vary in the Recent sediment but are uniform in the Miocene rock.

Amino acids may occur in both proteins and humic complexes in the Recent sediment, while they are probably only present in stabilized organic substances in the Miocene rock. Thermal decomposition of protein amino acids may be affected by surface catalysis in the Recent sediment. The apparent activation energy for the decomposition of alanine in this sediment is 8400 calories per mole. Yields of amino compounds from the heated sediments are not affected by thermal decomposition only.

Amino acids in sediments may only be useful for geothermometry in a very general way.

A better picture of the amino acid content of older sedimentary rocks may be obtained if these sediments are heated in a bomb with H_2O at temperatures around 150° C prior to HCl hydrolysis.

Leucine-isoleucine ratios may prove to be useful as indicators of amino acid sources or for evaluating the fractionation of these substances during diagenesis. Leucine-isoleucine ratios of the Recent and Miocene sediments and humic acids are identical. The humic acids may have a continental source.

The carbon-nitrogen and carbon-hydrogen ratios of sediments and humic acids increase with heating time and temperature. Ratios comparable to those in some kerogens are found in the severely heated Miocene sediment and humic acid.

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INTRODUCTION

With the development of sensitive chromatographic techniques for the separation and measurement of amino acids the way was opened for the search for amino acids in geologically old materials. Abelson (1954, 1955) discovered a number of amino acids in bones, teeth, and shells ranging in age from Devonian to Recent. Abelson (1954), Vallentyne (1957, 1964), and Poveledo and Vallentyne (1964) measured the thermal decomposition characteristics of various amino acids in pure aqueous solutions with the ultimate purpose of using this data for developing a geothermometer for sediments. Jones and Vallentyne (1960) analyzed Recent and Eocene lake sediments and used the data on the thermal decomposition of alanine to estimate the maximum continuous temperature to which the Green River formation was subjected during its history. They also heated Pleistocene shells in order to duplicate the amino acid composition found by Abelson in Miocene shells of the same species.

Hare (1962, 1963), Hare and Abelson (1964), and Lowenstam (1963) discuss the amino acid contents of mollusc shells of various ages and evolutionary development. They are concerned with the evolution of the protein matrix of molluscs and the effects this matrix has upon shell mineralogy. Needless to say, they are interested in the preservation of the amino acids through time in ancient shells.

Hydrolyzates of Recent and Pleistocene lake sediments were analyzed for amino acids by Kleerekoper (1957), Povoledo (1959), and Swain *et al.* (1959). The variation of amino acids in Recent marine

surface sediments and cores as a function of sedimentary environment was studied by Degens et al. (1961, 1963, 1964a). Also the variation of amino acids at various depths in the "Experimental Mohole" which penetrated essentially undisturbed Recent to Miocene sediments was studied by Rittenberg et al. (1963). A comparison between the amino acid spectra of Recent and Oligocene sediments of similar lithology and chemical composition was carried out by Erdmann et al. (1956). A large number of sedimentary rocks of all ages, some ranging as far back as the Pre-Cambrian, were analyzed for amino acids by Swain et al. (1958), Bajor (1960), Degens and Bajor (1960, 1962), and Jones and Vallentyne (1960). The amino acid content of Recent ocean water and of several oilfield brine waters of various ages as far back as the early Paleozoic was determined by Degens et al. (1964a,b).

Abelson (1954) found that only certain amino acids; viz., glycine, alanine, glutamic acid, leucine, valine, and aspartic acid were present in the vertebrate fossils he studied (the formulae for the amino acids discussed in this paper are provided for reference in Appendix I, page 130). Pyrolysis experiments performed on the alga Chlorella pyrenoidosa yielded paper chromatograms similar to those for Miocene shells estimated to be 25 million years old (Abelson, 1961). The major amino acid constituents of these samples were alanine, glutamic acid, glycine, isoleucine, leucine, and valine. Minor amounts of aspartic acid, lysine, proline, and tyrosine were also found. All of these analyses were performed using paper chromatography.

Jones and Vallentyne (1960) pyrolyzed Pleistocene Mercenaria mercenaria shells in open vessels under nitrogen and in evacuated

closed vessels. They hoped to produce an amino acid spectrum similar to that of Miocene Mercenaria shells. They found that all of the amino acids, when in the shell matrix, decomposed more rapidly than in pure aqueous solution. The shell material heated in the open vessels contained, after pyrolysis, significant amounts of aspartic acid, lysine, the leucines, and phenylalanine. Most of the other amino acids which were considered to be stable on the basis of the kinetics of thermal degradation of amino acids in pure aqueous solutions were not detected. The analyses were done using the column method of Moore and Stein (1954a,b).

Lowenstam (1963) reports analyses of unusually well preserved Pennsylvanian nautiloid shells of the species Pseudoorthoceras knoxense which yielded lysine, histidine, arginine, aspartic acid, tyrosine, serine, glutamic acid, glycine, and alanine.

Hare (1962) studied the amino acid contents of Mytilus californianus ranging in age up to 5460 years based upon carbon-14 measurements. He attributed the presence of cysteic acid and methionine sulfone to the presence of oxygen during the early diagenesis of the shells. He stated that the presence of oxygen and the effects of microbiological attack may account for the discrepancies between the results determined by Jones and Vallentyne (1960) from pyrolyzed Mercenaria shells and the amino acid contents of fossil Mercenaria shells. Hare also suggested that the differences between analyses he did on Recent shells up to 5000 years of age using the automatic recording amino acid analyzer and analyses done by Abelson on 1000 year old clam shells using paper chromatography were due to the

differences in precision of the two methods.

It has been argued that the relative stability of amino acids in the geological environment is a function of the thermal stabilities of the individual amino acids. Published data is available on the thermal decomposition of only a few amino acids in pure aqueous solution. Abelson (1954) determined the Arrhenius equation for alanine. The equation he obtained was $k = 3 \times 10^{13} e^{-44,000/RT}$ where k is the specific reaction rate constant for the thermal decomposition of alanine at any temperature, R is the gas constant, and T is the Kelvin temperature at which the reaction is being carried out. Vallentyne (1956) published similar data for phenylalanine. He obtained the equation, $k = 1.7 \times 10^{8} e^{-30,000/RT}$. Vallentyne later (1964) published equations for serine, threonine, and pyroglutamic acid which are $k = 4 \times 10^{9} e^{-29,350/RT}$, $k = 2 \times 10^{12} e^{-33,800/RT}$, and $k = 2 \times 10^{9} e^{-35,800/RT}$ respectively. If decarboxylation is the predominant reaction causing the thermal degradation of pyroglutamic acid, then subsequent hydrolysis yields gamma-aminobutyric acid. The work of Povoledo and Vallentyne (1964) showed that at elevated temperatures glutamic acid is in equilibrium with pyroglutamic acid. On the basis of the kinetic data for the decomposition of amino acids and the studies of fossils it was concluded that glycine, alanine, valine, isoleucine, leucine, and glutamic acid were among the more geologically stable amino acids while phenylalanine, serine, threonine, arginine, and histidine appear to be much less stable. Proline and lysine appear to be moderately stable while tyrosine, cystine, and methionine are

considered to be rather unstable. Thermal decomposition studies of aspartic acid in aqueous solution indicate that this amino acid is thermally unstable. However, it occurs in some very old fossils and in some pyrolyzed shell materials (Jones and Vallentyne, 1960, and Vallentyne, 1964). Essentially all of the amino acids known to occur in HCl hydrolyzates of proteins have been found in modern shell and bone material.

Analyses of Recent and fossil sediments have yielded amino acids in varying amounts. Recent sediment hydrolyzates from both freshwater and marine sediments have yielded essentially all of the amino acids found in HCl hydrolyzates of normal proteins. Recent marine sediments from nearshore basins yielded 3,000 to 5,000 ppm. of amino acids under oxidizing conditions and between 500 and 600 ppm. under reducing conditions according to studies by Degens and Bajor (1960) and Degens et al. (1961, 1963). One part per million is approximately equal to 10^{-2} micromole per gram sediment of amino acids. Recent marine sediments of red clay type collected at the top of the test Mohole drilling off Guadalupe Island in the Pacific yielded approximately one-tenth the quantity of amino acids found in nearshore basin sediments of the San Diego trough. Recent lake deposits have yielded approximately two-tenths of the amount of amino acids found in marine nearshore oxidizing sediments. All of the sediments referred to above are fine-grained clastic sediments. It appears evident that the greater abundance of amino acids in Recent marine nearshore oxidizing sediments is the result of biological activity in the sediments and that these amino acids are primarily peptide bound (Degens et al.,1963).

Fine-grained sediments of various geologic ages from the Pre-Cambrian to the Tertiary have yielded amino acid totals ranging from 28 to 450 ppm. (Swain et al., 1958; Erdmann et al., 1956; Bajor, 1960; Degens and Bajor, 1960, 1962; Jones and Vallentyne, 1960). There is considerable disagreement between the analyses with respect to the apparent geologic stability of several amino acids. The analyses were carried out by means of paper chromatography, column chromatography, or wet chemical methods.

Erdmann et al. (1956) compared the amino acid contents of a Recent and an Oligocene marine sediment of similar composition. Their work revealed only a few amino acids in both the Recent sample and the Oligocene sample. The Recent sample was a typical shallow-water marine deposit from the inner continental shelf of the Gulf of Mexico taken from a core depth of 120 cm. It was estimated to be only a few thousand years old. The total amino acid content measured was approximately 300 ppm. The amino acids found were valine, the leucines, alanine, glutamic acid, aspartic acid, glycine, proline, tyrosine, and phenylalanine. In the comparable Oligocene deposit which yielded alanine, glutamic acid, glycine, proline, the leucines, and aspartic acid only about 50 ppm. of total amino acids were found. At a core depth of 140 cm. in the San Diego Trough Degens et al. (1963) reported essentially all of the amino acids found in acid hydrolyzates of normal proteins. On the basis of radiocarbon data this portion of the core was estimated to be approximately 10,000 years old. At a similar depth in the reducing sediments of the Santa Barbara basin some of the amino acids were only present in trace amounts. Those found in greater than trace amounts

did not correspond to those determined by Erdmann and his associates.

Swain et al. (1958) analyzed Devonian deposits which yielded a limited selection of amino acids predominantly of the type with aliphatic sidechains. Jones and Vallentyne (1960) analyzed a portion of the Green River shale (Eocene) and obtained only glutamic acid, aspartic acid, gamma-aminobutyric acid, the leucines, and alanine.

Degens and Bajor (1960, 1962) measured the amino acids in a series of fine-grained sediments ranging in age from Pre-Cambrian to Tertiary in age. They report data on only eleven amino acids; i.e., aspartic acid, glutamic acid, serine, glycine, threonine, alanine, tyrosine, valine, lysine, arginine, and cystine. No data are provided on isoleucine, leucine, and several other amino acids although they were present. Among the amino acids found are aspartic acid, serine, threonine, tyrosine, and arginine which on the basis of thermal degradation kinetics and fossil data are considered to be relatively unstable amino acids. In some of the analyses the "unstable" amino acids are found in greater quantity than the "stable" amino acids. The "stable" amino acids include glutamic acid, alanine, valine, glycine, leucine, and isoleucine.

The unheated Recent sediment used in the present experiment yielded amino acids in amount comparable to those found by Degens and Bajor (1960) and Degens et al. (1963) in surface sediments of the North sea and of the San Diego Trough. The unheated Recent and Miocene sediments yielded all of the amino acids found in HCl hydrolyzates of normal protein.

NATURE OF THE PROBLEM

The disparities noted in the results of various workers who have studied amino acids in geologic materials may result, in part, from differences in the sensitivity of the methods used for analysis; they may actually reflect dissimilarities in the samples created by the effects of geologic time, temperature, or some other factor. It seems unlikely that the differences are due to unusual amino acid compositions in the original sediments or shells since modern counterparts of these materials contain the entire spectrum of protein amino acids. The fact that aspartic acid is found in many of the ancient samples throws some doubt upon the importance of thermal decomposition as the cause of the elimination of amino acids through geologic time. Vallentyne (1964) found that aspartic acid in pure aqueous solution is quantitatively converted to malic acid and ammonia at temperatures greater than 160°C. No data are provided concerning this reaction at temperatures less than 160°C; therefore, it is not possible to conclude that 160°C is a lower limiting temperature for this reaction. The occurrence of aspartic acid, serine, and tyrosine in unusually well preserved aragonite shells of the Pennsylvanian nautiloid Pseudoorthoceras knoxense is further evidence that the "unstable" amino acids may also be preserved for very long periods of time in mollusc shells. Vallentyne (1964) feels that the reported occurrences of the thermally unstable amino acids in rocks and fossils of greater geologic age may represent contamination. This may be the case; however, no systematic effort has been undertaken to demonstrate the effects of natural contamination on sediments or fossils. The absence of valine

in the Oligocene sediment (Erdmann et al., 1956) is also peculiar since it is the most abundant amino acid in the Recent counterpart of this sediment, and it is reported to be very stable geologically. The non-occurrence of this "stable" amino acid mitigates against the hypothesis of contamination since it is relatively abundant in proteinaceous material.

Recent developments in the methods of analysis for amino acids have made it possible to determine quantitative differences in the range of 10^{-8} to 10^{-12} molar concentrations of amino acids (Abelson, 1964). Of course, the precision at the lower concentrations is somewhat limited. However, the newer methods are generally more precise than paper chromatographic methods at the same concentration level since small amounts of dissolved electrolytes interfere with the separation of amino acids on filter paper. More precise concentration measurements can be produced using the recording photometer instead of the naked eye.

The results obtained thus far on the preservation of amino acids in ancient sediments are tenuous. It was felt that a comparison of the effects of heating Recent and Miocene marine sediments might indicate the importance of thermal decomposition on the amino acid spectra of sedimentary rocks.

In Recent sediments a large proportion of the amino acid content may be present as proteins, peptides, and free amino acids in the cells of micro-organisms and adsorbed loosely to the unstabilized organic matter and clays of the sediment. Other amino acids are proba-

bly present in the detrital organic material originally formed in terrestrial soils.

The organic fraction present in the older rocks may in part represent the material of immature sediments which is insoluble in the pore solutions that are present during early diagenesis. It may also represent the polymerization products of metastable constituents present in the original sedimentary organic matter. Amino acids fixed in such a stabilized structure would be expected to show different characteristics of thermal decomposition from those amino acids present in proteins, peptides, or in the free state.

SCOPE OF THE STUDY

Two sediment samples were chosen for comparison; viz., a Recent marine sediment from the San Pedro Basin and a sample of the Miocene Malaga mudstone collected from the sea cliffs at the northwest end of the Palos Verdes Peninsula. Both samples are from coastal Southern California.

The experiments consisted of heating the two sediments in a bomb at various temperatures and at a constant pressure of water. The experiment was designed in such a way as to yield data under ideal conditions which would be useful in measuring the kinetics of thermal decomposition of the amino acids in the two sediments. The measured amino acids are those available to 6 N HCl under reflux conditions at 100°C for 24 hours. Uniform conditions were maintained at all times in order to produce comparable results since it is known that varying the conditions of hydrolysis can affect the yields of amino acids from

sedimentary rocks.

Aliquots of the sediments were heated with water in a bomb at various temperatures ranging up to 500° C. The pressure of water was maintained as close to 500 bars as possible.

A second aliquot was heated up to the assumed minimum temperature of a previous run and for the same time as the pre-heating period of this run. Difficulties were encountered in duplicating the exact thermal histories from run to run even though uniform procedures were maintained throughout the investigation. The purpose of a second run was to determine the initial concentration of a sample run.

After heating, the samples were refluxed for twenty-four hours at 100° C with 6 N HCl, and the same procedures were followed in preparing the samples for analysis as described by Degens and Reuter (1963). The samples were analyzed on the modified amino acid analyzer built by Dr. Egon T. Degens at the Woods Hole Oceanographic Institute.

Unheated and heated samples of humic acids derived from both the sediments studied were analyzed for amino acids in order to compare their amino acid composition to those of the unheated and heated bulk sediments.

It is known that the alkali soluble-acid insoluble organic fraction of sediments, termed humic acids, contains a large proportion of the amino acids in sediments. Since the humic acids in solid form are precipitated by acidifying the sodium hydroxide solution used for extraction of the sediment it is possible that amino acids or peptides are adsorbed to the active surfaces of the precipitate rather than held as a structural part of the humic acid complex. The humic acid extracts

were refluxed for 24 hours with 6 N HCl and an aliquot heated up to the vicinity of 200°C. The aliquot which was heated was refluxed again with 6 N HCl in hopes that the severe treatment would indicate whether amino acids are present as a structural part of the humic acid complex.

Other aliquots of the humic acid extracts and of the two sediments were heated for four hours at two temperatures in order to test the effects of geochemical environment on the degradation of the amino acids. Appreciable yields were obtained from those samples heated in the vicinity of 200°C, but the samples heated up to 470°C yielded amino acids at levels similar to those found in blank runs.

SPECIMENS USED IN THE HEATING EXPERIMENTS

RECENT MARINE SEDIMENT

The Recent sediment was collected aboard the Allen Hancock Foundation oceanographic vessel Velero IV in May, 1963. It was collected by means of a three foot grab sampler at a depth of 823 meters in the San Pedro Basin between Catalina Island and the Palos Verdes Peninsula in Southern California. The location was Allen Hancock Foundation Station 8682 at latitude 33° 35' 40", Longitude 118° 22' 00". The sample was frozen aboard the ship and kept frozen except when being handled during the experiment.

The specimen represents a homogenate of the upper 25 to 30 centimeters of the sediment column. The specimen was collected from the top of the sedimentary material lowered upon a washing screen in order to get material as young as possible.

No attempt was made to measure the Eh or pH of the sample. Aerobic conditions prevailed as evidenced by the abundance of living organisms in the sediment. No macroscopic organisms were observed in the portion of the sample which was retained for this study. An account of the general environmental conditions in the offshore Southern California basin sediments as well as the mineralogical composition of the sediments is given by Emery (1960).

In order not to affect the amino acid composition the sample was air-dried in a fume hood before mixing. It was spread out in a

clean enameled pan and turned over frequently with a clean glass stirring rod in order to facilitate drying.

After the sample was thoroughly air-dried it was sieved. The minus 200 mesh fraction was retained for the heating experiments since it was believed that few macroscopic organisms would pass through this mesh. Also most of the detrital organic material characteristically found in nearshore sediments would be expected to be present in this fraction. The fine fractions would be expected to contain the geologically important organic material since the most abundant organic material in sedimentary environments is finely dispersed. To this extent this fraction is considered to be representative of fine-grained clastic sediments.

The sample when dried had a dark olive green color and resembled many of the fine-grained siltstones of late Tertiary age in Southern California.

The main consideration in the choice of this sample was availability. The number of amino acid analyses on Recent marine sediments are few. Oxidizing sediments were shown by Degens et al. (1963) to yield relatively large quantities of amino acids.

MIOCENE MALAGA MUDSTONE

The Malaga mudstone has been classified as a member of the Miocene Monterey shale (Woodring et al., 1946). It is predominantly a chocolate brown to olive gray massive radiolarian mudstone or fine-grained siltstone. Diatomite, diatomaceous shale, and tuff are the other lithologic units making up the member. Limy phosphatic nodules

are found in the mudstone.

The Malaga mudstone was primarily chosen for this investigation because of its age, and because it contains the highest percentage of nitrogen (0.325%) of any sedimentary rock in Southern California (Trask and Patnode, 1942). It is possible that this rock has a higher concentration of amino acids than any sediment of comparable age in Southern California.

Dr. Thane McCulloh of the U.S.G.S. estimates that the Malaga mudstone from the region of Malaga Cove on the Palos Verdes Peninsula has not been covered by more than 1000 feet of overburden based upon measurements which he has made on the porosities and bulk densities of this rock (personal communication). Assuming that the thermal gradient was similar to that of the present time the Malaga mudstone was probably not subjected to temperatures greater than 30°C during its history. The high organic carbon and nitrogen content of this rock may indicate that the original Miocene sediment was as rich in amino acids as the Recent sediment. The easily hydrolyzed (proteinaceous) amino acids may have been removed in pore solutions during early diagenesis and compaction or through biological activity.

When a rock is collected from the zone of weathering there is always danger of Recent contamination from organic matter in the soil above it. This is especially true for a rock which is not well cemented.

The sample used in this study was collected from the sea cliff above Malaga Cove at the northwest end of the Palos Verdes Peninsula in Southern California. It was located between twenty and twenty-five

feet above the base of the member and approximately forty feet above the beach. This is well above the portion of the cliff which is wet by the waves. The sample material retained for analysis was removed after digging for a distance of three feet into the cliff face.

The mudstone is a rather impermeable rock, however, at this locality it weathers out into pebble- to boulder-sized flat fragments. This is probably due to the presence of microfractures resulting from local deformation. The collecting site was chosen for the following reasons:

1.) it is easily accessible

2.) the section is rather well known in this area

3.) the cliff is very steep and can be expected to shed surface debris frequently

4.) seepage of water into the rocks is expected to be minimized because of the steepness of the cliff-face and the relative impermeability of the rock.

Dr. David Thurber of the Lamont Geological Observatory analyzed the sample for carbon-14. He could not detect any significant carbon-14 activity. The sensitivity of the counter used is such that it can only be stated that the apparent age of the sample is greater than 35,000 years. However, as far as Dr. Thurber could tell the sample was dead. The presence of radiocarbon activity should indicate the extent of contamination by Recent organic matter.

After collection the fragments were peeled with a clean knife to insure removal of any surface contamination and air-dried. They were then crushed in a clean porcelain mortar. The resulting fragments

were ground in a ball mill overnight to reduce their size further and to mix the sample more thoroughly. Heating of the sample during grinding was not a factor since the ball mill turned rather slowly. Also any increase in temperature which may have occurred was small compared to the temperatures used in the experiment. The sample was then sieved and the -200 mesh fraction was retained for use in the heating experiments. The fragments which did not pass the 200 mesh were crushed further in the mortar until essentially all of the material passed the fine sieve. Thin-sections reveal that this is a uniformly fine-grained rock.

HUMIC ACIDS

The following is a description of the procedure used in obtaining the humic acid extracts of both the Recent marine sediment and the Malaga mudstone. It is essentially the same as described by Anderson (1961).

Three hundred grams of the Miocene sediment and 36.5 grams of the Recent sediment were extracted using 0.3 N NaOH in aqueous solution. Five milliliters of solution were used for each gram of sediment. The slurry was stirred at room temperature for 24 hours. The bulk of the suspended particles were allowed to settle, and the supernatant was decanted. A volume of 0.3 N NaOH solution equal to half of the amount used initially was added to the sediment and the mixture was stirred at room temperature for an additional hour. The suspensions were centrifuged and the supernatants were combined. Concentrated hydrochloric acid was added until the pH was one. The solution was

permitted to stand until a black precipitate formed. After the precipitate was centrifuged its volume was reduced and much of its water was removed by alternate freezing and thawing. Final drying was done in a vacuum at 50° C.

The amounts of humic acid extracted from the two sediments are different as would be expected considering their respective ages. Approximately eight times as much humic acid is extractable from the Recent sediment as from the Miocene sediment. However, the organic carbon contents of the two sediments are 3.66% and 6.29% by weight respectively.

APPARATUS AND PROCEDURES USED IN HEATING EXPERIMENTS

HEATING CONTAINERS

The vessels used in the heating experiments were modified Morey-Ingerson bombs. Figure 1 shows a cutaway drawing of the bombs used in the experiment. The material of the bomb is inconel X, a stainless alloy with a low thermal coefficient of expansion.

The bomb was sealed by placing a pure gold disc 0.005" thick between the piston and the shoulder surrounding the top of the sample chamber and turning the cap down with a long-handled wrench while the body of the bomb was held in a vise. Flats were machined on the cap and body of the bomb to provide a grip for the wrench.

The bomb itself was used as the sample container. Its internal capacity was 19.2 cubic centimeters. The pressure was controlled by the amount of water placed in the bomb with the sample. The control of pressure is described below.

A thermocouple well is located at the external end of the piston.

FURNACES

Two cylindrical resistance furnaces mounted horizontally were used in the heating experiments. Each furnace is constructed of an alundum tube 60.9 centimeters long with an inside diameter of 7.6 centimeters and an outside diameter of 9.7 centimeters. The tubes are open at both ends. These tubes are wound with 28.3 meters of #17 D. H. Nichrome V Wire. The windings are pitched 0.411 centimeter per turn

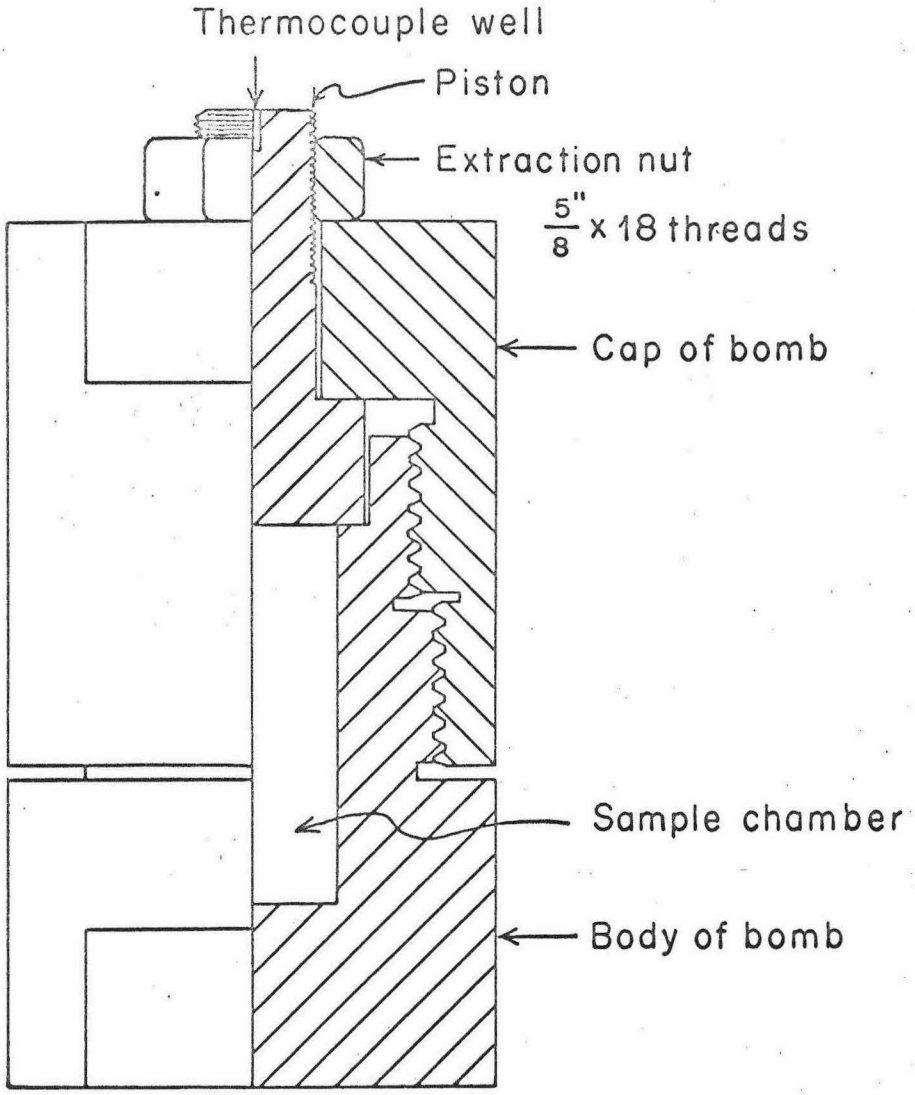

Fig. 1

Drawing of bombs used in heating experiments.
Scale 1:1
Sample chamber has a volume of 19.2 $cm.^3$
A .005" gold disc was used as a seal between the piston and the body of the bomb. Bomb was made of Inconel X.

near the ends and 0.826 centimeter per turn near the center. There are 90 turns in all and the tube is wound for 55.9 centimeters of its length. The wire has an overall resistance (cold) of 30 ohms. Operating voltage was 220 volts A.C. The tube is wrapped with 10.4 centimeters of insulation and aluminum sheeting and bound with steel straps.

TEMPERATURE CONTROL AND MEASUREMENT

The temperature of the furnaces was controlled by two Minneapolis-Honeywell Pyrovane controllers. Each controller was activated by a chromel-alumel thermocouple whose junction was mounted as close as possible to the position occupied by the bomb. The thermocouple junction was mounted in the center hole of a disc of steel in order to keep it from contacting the wall of the furnace. The controller thermocouple leads came out of the rear of the furnace in order not to disturb the thermocouple each time a run was made.

To reduce temperature fluctuations caused by air circulation and heat loss at the ends of the furnace the following steps were taken;

1.) each furnace was mounted horizontally beneath a workbench in a room where air circulation was low
2.) the rear of each furnace was covered with insulating material and forced against a wall
3.) the front of each furnace was covered with firebrick leaving an opening only for the thermocouple leads
4.) a large, heavy baffle was forced against the firebricks to hold them in place.

The temperature was measured by reading the potential developed between the hot junction of a chromel-alumel thermocouple placed in the thermocouple well of the bomb and the cold junction of the same thermocouple placed in an ice-water mixture. The potential was measured on a Leeds and Northrup potentiometer.

The temperature measured was that of the outer portion of the bomb. It would have been almost impossible to seal a thermocouple in the bomb and expect to obtain the same conditions as those which prevailed during an actual run. The thermal conductivity of the metal of the bomb is rather high. For these reasons the temperature measured by the thermocouple was assumed to hold for the sample chamber as well.

The bomb was lying on its side when in the furnace. Therefore, one side was closer to the wall of the furnace than the other and actually in contact with it over an infinitely small area. This would be expected to create a temperature gradient across the bomb and also across the sample chamber. It was assumed that the temperature measured by the thermocouple closely duplicated the average temperature of the sample chamber because it was centrally located with respect to the sample chamber; the high thermal conductivity of the bomb also would favor this. The measured temperature probably did not differ from the actual temperature by more than 5° C.

At a temperature of approximately 470° C the maximum temperature fluctuation was 2° C; at 200° C the maximum temperature variation was 4° C. The heating cycle was twice as long at 200° C than at 470° C. The lower temperature corresponded to the time when the controller started the furnace on a new heating cycle, and the higher temperature

corresponded to the time when the bomb had absorbed as much heat as possible under the prevailing conditions. The temperature fluctuations within the bomb were probably less because of the high thermal inertia of the bomb.

HEATING EXPERIMENT PROCEDURES - PRESSURE CONTROL

A sediment sample weighing 5.00 grams was transferred to a dry bomb. The volume of distilled water calculated to produce a pressure of 500 bars at the expected temperature of the run was pipetted over it. The distilled water was previously boiled and cooled quickly in a freezer in order to remove as much atmospheric oxygen as possible. The values for the specific volume of water at various temperatures were taken from the data of Kennedy (1950). After the water was added the bomb was agitated to make certain the sample was wet and to remove air bubbles. A stream of purified nitrogen was directed at the surface of the sediment-water slurry and held there for several minutes to allow the nitrogen to displace as much atmospheric oxygen as possible. In order to prevent splashing and to reduce the danger of contamination, the nitrogen was not bubbled through the liquid.

The liquid was visibly agitated by the stream of nitrogen gas. A gold disc was placed over the sample chamber and the tube carrying the nitrogen was withdrawn simultaneously. The cap of the bomb was quickly screwed into place and tightened in order to form the seal.

The bomb was placed in a pre-heated furnace and the thermocouple junction inserted. Care was taken to locate the bomb at the same position in the furnace each time in order that the thermal

history would be similar for each run. After the furnace was covered the bomb temperature was measured at intervals. The spacing of the intervals was closer when the bomb temperature was nearing the expected temperature of a run. The time at which the bomb temperature reached 5° below the maximum temperature was considered to be the beginning of the run. After the bomb reached its maximum temperature its temperature was monitored occasionally during a run.

When a run was completed the bomb was removed from the furnace with the thermocouple still in it. The bomb was cooled with a stream of compressed air. Cooling was continued until the temperature measured by the thermocouple was less than 90° C. The bomb was then placed in a freezer. The same procedure was followed from run to run. It was assumed that the same quantity of amino acids destroyed during cooling of a sample run was destroyed during the cooling of a second run. To reduce the danger of contamination and to protect the bombs from thermal shock the bombs were not quenched in water.

The bombs were opened after the contents were frozen solid. This precaution was taken since gas developed in the bomb during heating. Foaming caused loss of part of the sample when the contents of the bomb were still liquid. As soon as the bomb's contents thawed they were transferred to a clean 500 ml. round-bottomed flask via a large clean funnel. A measured amount of triple-distilled water was used in the transfer and for rinsing the sample chamber of the bomb. Every precaution was taken to prevent contamination of the sample during this transfer. A quantity of concentrated hydrochloric acid calculated to produce a final concentration of 6 N HCl was added. The flask was put

in a boiling water bath and the sample was refluxed for 24 hours. The procedure used in preparing a sample for analysis is described in Appendix II.

In order to get the initial amino acid content of a sample run, a second run was made. An attempt was made to duplicate the thermal history of the previous run during heating and cooling. The same bomb was used, and it was placed at the same location in the furnace. The furnace controller setting was unchanged. The bomb was put in the furnace for the second run at the same point in the heating cycle as for the first run. The time that the furnace was open during which the bomb and thermocouple were positioned is the only variable that was not exactly duplicated from run to run. The heat loss during this interval may have caused the difference between the thermal histories of successive runs. If the second run attained a temperature within three to five degrees of the first run after the same pre-heating time, it was aborted; its amino acid concentration was used as the initial concentration of the first run.

Despite every precaution it was not possible to predict the maximum temperature of a run to better than 5°C. This made it difficult to attain the desired pressure exactly. When the furnace controller was set at a new temperature, a trial run was made without a sample in order to get an approximate value for the maximum temperature at the furnace setting. Using the available volume of the bomb (volume of sample chamber minus volume of sediment) and the specific volume of water at the expected maximum temperature, the amount of water needed to produce the desired pressure (500 bars) was calculated. This amount

of water was added to the sediment in the bomb. The maximum temperature actually achieved did not always correspond to the expected temperature. Also, as mentioned above, the temperature fluctuated slightly during the run.

Values calculated for the density of water in the bomb based on the amount of water added and the available volume of the bomb indicate that the pressure varied as much as 50 bars at the lower temperatures and 10 bars at the higher temperatures.

Several gaseous compounds were produced during the heating experiments all of which contributed to the total pressure within the bomb. It is impossible to assess the pressure effect these gases produced. The effective total pressure during a run is the poorest known variable in the heating experiments.

OBSERVATIONS

Marked physical and chemical changes were noted in the samples after heating. This was particularly evident in samples heated at higher temperatures. The Recent sediment and the Malaga mudstone samples each have a greenish brown color, although the Recent sediment is greener than the Malaga mudstone. Both samples are loose, fine powders. At the lower temperatures no noticeable changes took place in the color or texture of the sediments. However, at the higher temperatures marked changes took place in the appearance of the samples. Both samples became black. A large proportion of the particles of the sample became loosely aggregated into fine gravel-like lumps. The lumps were friable but were not broken by vigorous agitation of the

suspending liquid. These changes occurred despite the fact that water had not leaked from the bomb.

A clear, apparently crystalline, substance was noted in the sediment residue of the Malaga mudstone when heated at higher temperatures. This substance was obviously produced during the experiment since the size of the crystals was much greater than the particle size of the original powder. Also the crystals included particles of the altered sediment. It was not possible to separate enough of this material for x-ray diffraction studies.

Some reaction took place with the walls of the bomb at higher temperatures. This was evidenced by thin flakes of material which adhered to the walls. A semi-quantitative emission spectroscopic analysis of this material showed that it was high in nickel and chromium, two of the metals of the inconel X alloy. Minor constituents included iron, titanium, silicon, and aluminum. The latter two are abundant in the silicate portion of the sediments.

The lower temperature samples smelled strongly of hydrogen sulfide when the bombs were opened. The Recent sediment seemed to produce more of this substance than the Malaga mudstone. The higher temperature samples gave off a strong odor of phenol when opened. The odor seemed to be stronger from the Malaga mudstone. Also the Malaga mudstone seemed to produce this odor at a lower temperature than the Recent sediment. When the Recent sediment was heated at lower temperatures the stronger smell of hydrogen sulfide may have masked the phenolic odor. After refluxing with 6 N HCl all of the heated sediments had the odor of phenol.

After most of the odors from the gaseous products had dissipated, the residues transferred to the refluxing flasks had a petroleum-like odor. Also an oily film was frequently noted on the surface of the water in the bomb.

SUMMARY

An attempt was made in these experiments to duplicate the geological conditions which might prevail during diagenesis or low temperature metamorphism. In order to produce measurable changes in a short time it was necessary to use higher temperatures than those which ordinarily occur in the sediment column during diagenesis. The sediments are probably saturated with water under natural conditions. The presence of the metal surfaces of the bomb may affect chemical reactions taking place during the heating experiments by catalyzing thermal decomposition reactions. However, this probably only applies to substances which become dissolved during the heating experiment. Any organic constituent which is part of a solid organic complex would probably not be affected by surface catalysis at the walls.

Most of the variation in the pressure during the experiment may be attributed to the fact that the experiments were conducted at temperatures below the critical point of water. Also, the quantity of gaseous and volatile products which added their partial pressures to the total pressure of the bomb was unknown.

Changes in pH conditions or oxidation potential which occurred during a run cannot be evaluated easily. Such changes must have occurred since substances like phenol and hydrogen sulfide, among

others, were liberated during the experiments. The acidic properties of these substances are well known to chemists.

It was evident from the appearance of phenol in the heating experiments that extensive breakdown of the organic fraction in the sediments had occurred. Organic geochemists and soil scientists have demonstrated the presence of substituted phenols and phenolic acids in sea water, sediments, and soil organic matter (Degens et al., 1964; Morrison, 1958). Lignins subjected to alkali fusion and dry distillation at similar temperatures to those used in the heating experiments have yielded products which contain abundant phenols and phenolic acids. Among those phenolic constituents derived from lignins by the methods described above are pyrocatechol, veratrol, eugenol, vanillin, guaiacol, phenol, ortho- and para-cresol, xylenol, catechol, 1-vinyl-3-methoxy-4-hydroxybenzene, and 1-propyl-3-methoxy-4-hydroxybenzene. Carboxylic acids of some of these substances have also been found. However, alkali fusion of pure cellulose has reportedly also given rise to phenolic substances (Brauns, 1952). To date no phenols have been reported in algae but some fungi have yielded phenolic compounds (Farmer and Morrison, 1964). The presence of phenol as a thermal degradation product of the organic matter of both the Recent and Miocene sediments suggests that phenolic compounds are important in nearshore marine sedimentary organic matter. These phenolic constituents may be derived ultimately from the lignin fraction of terrestrial plants (Kononova, 1961). However, not enough information is available on primary marine sources of phenolic compounds or the synthesis of such compounds from other organic constituents during the early diagenesis

of sediments to say with certainty that the phenols in the marine sediments are land derived. Systematic thermal degradation experiments at different temperature on these two rocks, or other rocks with widely different organic contents, may help to reveal the relationship between phenolic constituents and the kerogenous organic fraction of sediments. It is planned to determine qualitatively the nature of the phenolic compounds present in the final concentrates from the heated sediments of the present experiments.

The significance of the thermal degradation of the organic fraction of the two sediments will be discussed along with the results of the amino acid analyses.

EVALUATION OF THE DATA

Problems encountered in trying to reproduce exact thermal histories from run to run make it difficult to assess the reproducibility of amino acid measurements from sample to sample. However, where comparable data is available the results are reasonably consistent considering the many complicating factors involved in experiments using impure natural materials. The following is a discussion of several of the major problems peculiar to this experiment which lower the precision of the amino acid measurements.

Analyses of protein hydrolyzates on an amino acid analyzer of the type used in this experiment yield data, under ideal conditions, which have a precision better than 3%.

Sedimentary rocks contain an entire spectrum of inorganic and organic constituents, some of which are extremely difficult to separate from the amino acids. Since ion exchange methods do not separate all the impurities from the amino acids it follows that these substances may interfere with their movement on the analyzer column. Thus the uncertainties in the measurement of amino acids in sediments may be expected to be larger than those of protein hydrolyzates. In this experiment the amount of sample material and the available analyzer time was limited; therefore, it was not possible to repeat analyzer runs on all of the samples. Where more than two analyzer runs are available on the same sample the calculated amino acid concentrations do not deviate from their mean value by more than ±10% except for the amino acids which are difficult to separate completely. See below. This

applies to individual amino acid concentrations with a mean value greater than 0.010 micromole per gram of sediment. In many cases the uncertainty is less than ±5%. It may be somewhat more than 10% for groups of amino compounds like threonine and serine or tyrosine, phenylalanine, and glucosamine. These groups of amino compounds are inherently more difficult to separate than others. Those amino compounds in the sediment samples which are separated on the column eluted with the more basic buffer do not separate as well as most of the other amino acids. This applies to the accelerated procedure used at the Woods Hole laboratory. The deviation from the mean for the amino compounds which are more difficult to separate is as high as ±15% in some samples at concentrations greater than 0.010 micromole per gram of sediment. All the amino acids present in quantities less than 0.010 micromole have average percentage deviations from the mean of less than ±20%.

The following two factors may be involved in producing these uncertainties:

1.) colored substances present in the sample may absorb light at the wavelengths used for measuring the amino acid concentration (440 and 570 millimicrons) and thus raise the background level

2.) aluminum hydroxide and other substances may travel with the amino acids on the ion exchange column competing for the exchange sites; this would be expected to decrease the separation efficiency and cause spreading of the peaks.

The effects described above were noted in some of the chromatograms. The increase in background and partial overlapping of peaks causes some uncertainty in the computation of the H x W constants for the unknowns (see Appendix II, page 132). Errors in the addition of sample solutions to the column may be discounted since the amino acid concentrations for a particular run would all be off in the same direction. This does not appear to be the case. One possible source of error may be the addition to the ion exchange column of suspended colloidal material in the samples.

A dark brown to black residue was seen to develop in many of the sample vials after they remained in the refrigerator a few days. This residue if stirred up required several hours to settle. Some of these residues were separated, washed, and re-hydrolyzed in order to determine whether their formation affected the measured amino acid concentrations in the sample. The accumulations were soluble in 6 N HCl. It is possible that the precipitate is a reaction product of phenolic constituents and amino acids or other organic substances remaining in the sample. Therefore, the hydrolyzates were extracted with ethyl acetate before they were analyzed. Ethyl acetate is immiscible with water and is a good solvent for the phenolic acids. The results obtained are in Table 4-1, page 34.

It can be seen from the table that for the higher temperature samples the total amount of amino acids in the residue is as great or greater than the total measured in the sample itself. The data for the lower temperature runs shows that the residue does not affect the total amino acid concentration by more than 2%.

Glycine is by far the most abundant amino acid in the precipitates. This may account for its variability in some of the sample runs. The more abundant amino acids in the residues are in general also the more chemically reactive. Threonine and serine, which are essentially absent or are not above the contamination level as measured in the sample solutions, are quite abundant in the residues.

Table 4-1. APPROXIMATE TOTAL MICROMOLES OF AMINO ACIDS FROM RESIDUES FORMED IN SAMPLE FLASKS COMPARED WITH TOTAL MICROMOLES MEASURED IN SAMPLE SOLUTIONS.

Sample Number	Max. Temp.	Total Time (Min.)	Total Amino Acids in Sample	Total in Residue	Percentage in Residue
Recent Marine Sediment					
A-13	198°C	240	69.34	1.03	1.5%
A-15	450°C	117	traces	0.036	--
A-14	473°C	240	0.061	0.021	34.4%
Recent Humic Acid					
C-2	475°C	240	traces	0.25	--
Malaga Mudstone					
B-21	465°C	88	0.026	0.011	42.3%
Miocene Humic Acid					
D-2	193°C	240	1.554	0.031	1.9%

The amino acids found in the residues are listed in order of decreasing abundance.

1. glycine
2. glutamic acid
3. serine
4. alanine
5. threonine
6. aspartic acid
7. leucine
8. isoleucine
9. valine
10. tyrosine
11. phenylalanine

If some of the residues were included with the solution added to the column, erratic results might be obtained since the residues are acid soluble.

Samples B-8 and B-13 are two samples of the same sediment which had similar thermal treatment. Comparison of the yields gives some idea of the reproducibility, from sample to sample, for the various amino acids. Glycine, alanine, phenylalanine, and tyrosine show the greatest range of variation. It is noteworthy that their peaks were not as well separated or as sharp as those of the other amino acids.

Duplicate samples of the unheated Malaga mudstone were analyzed to determine the reproducibility of the original amino acid concentrations. A large broad peak was observed to underlie the early peaks on the chromatograms for each of these aliquots. However, it was possible to get two good runs for one of the aliquots in which the early peaks were satisfactorily separated. The early peaks did not separate well on the chromatogram of the second aliquot of sediment. The peaks affected were those for aspartic acid, threonine, serine, glutamic acid, and proline. The peaks for glycine, alanine, valine, leucine, and beta-alanine yielded measured values which varied less than 5.0%; isoleucine varied less than 10.0%. The totals of tyrosine, phenylalanine, and glucosamine were compared since they did not separate. The totals showed a variation of less than 5.0%. The values accepted for the early peaks of B-1 are those from the aliquot producing the better chromatogram. They include aspartic acid, threonine, serine, glutamic acid, and proline. The values taken for glycine, alanine, valine, isoleucine, leucine, beta-alanine, and the totals of tyrosine,

phenylalanine, and glucosamine represent the averages for both aliquots. In the heated samples the interfering peak was not visible. Since glucosamine was largely destroyed in the heated samples the peaks of phenylalanine and tyrosine became measurable after heating.

The variation in the measured values for the humic acid extracts are less than 15.0% for all of the amino acids measured.

LABORATORY CONTAMINATION

Control experiments were performed in which a quantity of triple-distilled water was heated in a bomb and carried through the complete extraction procedure. The same quantity of reagents and distilled water was used as were utilized in a normal analysis. Three such blank runs were made at different times. The maximum value obtained for individual amino compounds has been subtracted from each of the sample runs. Table 4-2 lists the maximum values for the amino compound concentration in the total blank. Also shown is the maximum contamination per gram of sediment sample.

Table 4-2. LABORATORY CONTAMINATION AND AMINO COMPOUND CONTRIBUTIONS FROM REAGENTS IN MICROMOLES PER TOTAL ANALYSIS AND PER GRAM OF SEDIMENT.

Amino Compound	Micromoles Total Analysis	Micromoles Gram of Sediment
cysteic acid	0.085	0.017
taurine	0.003	0.000
urea	0.790	0.160
aspartic acid	0.018	0.004
threonine	0.001	0.000
serine	0.001	0.000
glycine	0.035	0.007
alanine	0.011	0.002
leucine	0.014	0.003
methionine	0.004	0.001
glutamic acid	0.002	0.000
beta-alanine	0.007	0.001

EFFECTS OF HEATING IN THE PRESENCE OF WATER ON THE CONCENTRATIONS OF AMINO SUBSTANCES IN SEDIMENTS

One of the primary goals of this experiment is to determine the effects of heating on the degradation of the 6 N HCl leachable amino acids in sediments. Only the amino compounds eluted from the analyzer column along with the acidic and neutral amino acids will be discussed in detail.

MEASURED AMINO ACID CONCENTRATIONS IN THE UNHEATED SEDIMENTS

Recent Marine Sediment

Table 5-1, page 39, shows the measured yields of the acidic and neutral amino acids from the Recent and Miocene marine sediments. The unheated sample, A-1, contains all of the amino acids known to occur in 6 N HCl hydrolyzates of normal proteins. It also contains some amino compounds which are generally not found in protein hydrolyzates. Among these amino compounds are beta-alanine, glucosamine, and gamma-aminobutyric acid. All of the amino acids except cystine, methionine, and gamma-aminobutyric acid are obtained in quantities ranging from 1.0 to 10.0 micromoles per gram of sediment. Cystine, methionine, and gamma-aminobutyric acid are found in concentrations less than 1.0 micromole per gram of sediment. It appears possible that beta-amino-isobutyric acid may be present; a clear peak corresponding to this amino acid was not obtained. This amino acid was detected in many of the heated samples. Another compound which has a very similar R_f value to that of hydroxylysine is present. Histidine also appears to be

Table 5-1. RELATIVE ORDER OF ABUNDANCE OF AMINO ACIDS IN THE UNHEATED SEDIMENTS USED FOR THE HEATING EXPERIMENTS.

Yields are expressed as micromoles per gram of sediment.

A-1 Recent Marine Sediment	Yield	B-1 Miocene Malaga Mudstone	Yield
glycine	9.10	tyrosine + phenylalanine + glucosamine	0.981
alanine	4.57	beta-alanine	0.633
proline	3.73	alanine	0.214
beta-alanine	2.98	leucine	0.154
serine	2.87	glycine	0.141
valine	2.46	valine	0.117
glutamic acid	2.32	isoleucine	0.096
phenylalanine	2.31	threonine	0.092
leucine	2.29	lysine	0.087
threonine	2.26	arginine	0.077
aspartic acid	1.69	proline	0.067
lysine	1.62	serine	0.039
isoleucine	1.41	methionine	0.035
arginine	1.36	glutamic acid	0.033
glucosamine	1.04	ornithine	0.030
tyrosine	1.01	one-half cystine	0.019
gamma-aminobutyric acid	0.54	aspartic acid	0.009
ornithine	0.41		
methionine	0.32		
one-half cystine	0.31		

present although its peak is interfered with by the large ammonia peak which follows it. An unknown found in very low concentration may be ethanolamine.

Except for glutamic acid, aspartic acid, and beta-alanine the relative concentrations of amino acids are similar to those measured by Degens et al. (1963) at the top of a core obtained from the San Diego Trough.

Miocene Malaga Mudstone

The concentrations of the amino acids obtained from the unheated Malaga mudstone are from one to two orders of magnitude lower than those found in the Recent sediment. See sample B-1 in Table 5-1 on the preceding page. The relative order of abundance differs considerably from that of the Recent sediment. It is interesting that serine and aspartic acid, which are considered to be thermally unstable, are found much lower in the list for the Miocene sediment than for the Recent sediment; however, glutamic acid and proline, which are considered thermally stable, are also found in relatively low concentrations.

All of the amino acids found in protein hydrolyzates are found in this sediment. Tyrosine, phenylalanine, and glucosamine are totaled because they did not separate on the analyzer column; most of the peak area can be attributed to glucosamine. These amino acids separated in most of the thermally treated samples. Beta-aminoisobutyric acid and gamma-aminobutyric acid may be present in moderate amounts. They did not separate well enough from other peaks, which

normally occur near them, to permit measurement of their yields.

THE VARIATION OF AMINO ACID CONCENTRATIONS PRODUCED BY HEATING SEDIMENTS AT SEVERAL TEMPERATURES

Recent Marine Sediment

Comparison of the amino acid concentrations measured in coupled runs reveals that the amino acids generally decrease in this sediment when heated for increased periods of time at the same temperature. The term, coupled runs, refers to the two runs used to determine initial and final concentrations of amino acids in sediments heated in a bomb. Since the bomb requires appreciable time to attain its maximum temperature, it is necessary to make a second run to determine the concentrations at the time the reaction temperature is achieved. For the yields of amino compounds from the heated aliquots of the Recent marine sediment see Table 5-2 on pages 42, 43, and 44.

In a few instances the final measured concentration is significantly higher than the initial concentration. An examination of the values for glycine and alanine in samples A-2 and A-3, heated at temperatures in the range of 157°-162°C, shows that these amino acids increase with time of heating. The analyses of samples A-5 and A-13, heated in the range of 195°-200°C, indicate that glutamic acid and alanine have increased as a result of prolonged heating. Aspartic acid, valine, and glucosamine show very little change. Changes in yields with time for the samples heated around 240°C will be described in a later section.

Table 5-2. CONCENTRATION OF ACIDIC AND NEUTRAL AMINO ACIDS AND AMINO SUGARS IN UNHEATED AND HEATED ALIQUOTS OF A RECENT MARINE SEDIMENT (micromoles per gram of sediment). The sample numbers are explained in Table III-1 in Appendix III.

L = probable low value.

Sample Number	Aspartic Acid	Threonine	Serine	Glutamic Acid	Proline	Glycine
A-1 (unheated)	1.69	2.26	2.87	2.32	3.73	9.10
A-2	0.990	2.11	1.99	1.66	2.67	4.44
A-3	0.616	1.51	1.45	1.15	2.50	6.56
A-4	0.192	1.61	0.950	0.780	2.35	5.37
A-5	0.031	0.060	0.046	0.323	1.29	1.77
A-6	0.085	--	--	0.596	1.32	0.492
A-7	0.009	*	0.004*	1.18	0.154	0.182
A-8	--	--	--	0.695	1.36	1.57
A-9	--	--	--	0.259	0.612	0.238
A-10	--	--	--	0.874	0.086	0.080
A-11	--	--	--	0.135	1.01	0.121
A-12	0.009	0.009	0.016	0.077	0.034	0.022L
A-13	0.034	0.339	0.132	0.273	1.66	3.37

* The quantity so marked represents the sum of the amino acids marked by an asterisk in the same sample.

Continued on next page.

Table 5-2 (continued). CONCENTRATION OF ACIDIC AND NEUTRAL AMINO ACIDS AND AMINO SUGARS IN UNHEATED AND HEATED ALIQUOTS OF A RECENT MARINE SEDIMENT (micromoles per gram of sediment). The sample numbers are explained in Table III-1 in Appendix III.

L = probable low value.

Sample Number	Alanine	One-half Cystine	Valine	Methionine	Iso-leucine	Leucine
A-1 (unheated)	4.57	0.307	2.46	0.320	1.41	2.29
A-2	3.46	0.195	2.11	0.189	1.22	1.75
A-3	3.92	0.111	1.80	0.098	1.04	1.50
A-4	2.96	--	1.74	0.140	1.11	1.38
A-5	2.09	0.217	0.918	--	0.333	0.565
A-6	1.24	0.217	0.857	--	0.331	0.620
A-7	0.122	--	0.051	--	0.138	0.006
A-8	1.78	0.257	0.767	--	0.295	0.538
A-9	0.542	0.084	0.326	--	0.144	0.161
A-10	0.043	0.046	0.097	0.013	--	0.018
A-11	0.189	0.015	0.464	--	0.125	0.126
A-12	0.024L	--	0.003L	--	0.006L	0.009L
A-13	1.70	0.080	1.07	0.060	0.580	0.902

Continued on next page.

Table 5-2 (continued). CONCENTRATION OF ACIDIC AND NEUTRAL AMINO ACIDS AND AMINO SUGARS IN UNHEATED AND HEATED ALIQUOTS OF A RECENT MARINE SEDIMENT (micromoles per gram of sediment). The sample numbers are explained in Table III-1 in Appendix III.

L = probable low value.

Sample Number	Tyrosine	Phenyl-alanine	Beta-alanine	Glucos-amine	Galactos-amine	Gamma-amino-butyric acid
A-1 (unheated)	1.01	2.31	2.98	1.04	--	0.544
A-2	0.522	1.04	1.17	0.620	--	0.736
A-3	0.448	1.07	0.799	0.241	--	0.420
A-4	0.536	0.812	0.672	0.452	0.348	0.648
A-5	0.203	0.286	0.198	0.136	0.030	0.342
A-6	0.254	0.315	0.108	0.009	0.010	0.413
A-7	0.015	0.002	0.019	--	--	0.786
A-8	0.218	0.260	0.165	0.064	0.021	--
A-9	0.067	0.084	0.038	--	0.002	0.385
A-10	--	--	--	--	--	0.520
A-11	0.033L	0.033L	0.050	--	--	0.521
A-12	--	--	0.009L	--	--	0.258
A-13	0.354	0.519	0.229	0.123	0.077	--

Miocene Malaga Mudstone

Comparison of coupled sample runs reveals that increased yields of various amino acids are obtained by heating for longer periods of time at the same temperature. The analyses given in Table 5-3 on pages 46, 47, and 48 show the yields of amino acids from the various heated samples of the Malaga mudstone. Comparison of samples B-2 and B-3 indicate that around 150°C all of the amino acids have increased significantly. Samples B-4 and B-5 show that glycine, alanine, valine, isoleucine, tyrosine, phenylalanine, glucosamine, and beta-alanine increase significantly when the sediment is heated in the temperature range of 153° to 158°C. Leucine shows no apparent change. In sample B-3 the yields of serine, proline, glycine, alanine, valine, isoleucine, and leucine approach or exceed their values in the unheated sediment; glycine, alanine, and valine are obtained in greater yields from aliquot B-5 than from the unheated Miocene rock. At 192° to 197°C only alanine seems to increase (sample B-6 and B-7).

The variations in the amino acid yields from the Malaga mudstone heated in the range of 235° to 242°C are discussed in a subsequent section.

Table 5-3. CONCENTRATION OF ACIDIC AND NEUTRAL AMINO ACIDS AND AMINO SUGARS IN UNHEATED AND HEATED ALIQUOTS OF THE MIOCENE MALAGA MUDSTONE (micromoles per gram of sediment). The sample numbers are explained in Table III-1 in Appendix III.

Sample Number	Aspartic Acid	Threonine	Serine	Glutamic Acid	Proline
B-1 (unheated)	0.0085	0.0924	0.0386	0.0328	0.0673
B-2	0.0035	0.0319	0.0190	0.0139	0.0567
B-3	0.0062	0.0392	0.0383	0.0163	0.0950
B-4	0.0073	0.0439	0.0212	0.0105	0.0648
B-5	0.0037	0.0372	0.0150	0.0079	0.0480
B-6	--	0.0173	0.0136	0.0127	0.0500
B-7	--	--	0.0056	0.0092	0.0377
B-8	--	--	--	--	--
B-9	--	0.0021	0.0041	0.0036	0.0254
B-10	--	0.0027	0.0053	0.0500	0.0244
B-11	0.0028	0.0016	0.0028	0.0015	0.0276
B-12	0.0014	*	0.0015*	0.0030	0.0128
B-13	--	--	--	0.0259	0.0419
B-14	0.0011	--	0.0063	0.0694	0.0416
B-15	0.0016	--	0.0133	0.0302	0.0312
B-16	--	--	--	0.0218	0.0192
B-17	0.0072	0.0627	0.0110	0.0283	0.0820
B-18	--	0.0085	0.0085	0.0065	0.0407
B-19	0.0013	0.0016	0.0022	--	0.0202
B-20	--	--	--	0.0032	0.0040

* The quantity so marked represents the sum of the amino acids marked by an asterisk in the same sample.

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Table 5-3 (continued). CONCENTRATION OF ACIDIC AND NEUTRAL AMINO ACIDS AND AMINO SUGARS IN UNHEATED AND HEATED ALIQUOTS OF THE MIOCENE MALAGA MUDSTONE (micromoles per gram of sediment). The sample numbers are explained in Table III-1 in Appendix III.

Sample Number	Glycine	Alanine	Valine	Iso-leucine	Leucine
B-1 (unheated)	0.141	0.214	0.117	0.0961	0.154
B-2	0.119	0.180	0.0856	0.0644	0.0979
B-3	0.231	0.224	0.118	0.1256	0.1475
B-4	0.104	0.150	0.102	0.0782	0.1210
B-5	0.150	0.218	0.135	0.0848	0.1210
B-6	0.159	0.236	0.0990	0.0760	0.113
B-7	0.131	0.255	0.092	0.062	0.093
B-8	0.103	0.257	0.0916	0.0497	0.0830
B-9	0.177	--	0.0552	0.0230	0.0438
B-10	0.167	0.238	0.0530	0.0255	0.0392
B-11	0.0505	0.179	0.0649	0.0416	0.0676
B-12	0.0343	0.0896	0.0311	0.0077	0.0135
B-13	0.160	0.312	0.0948	0.0536	0.0864
B-14	0.202	0.259	0.0602	0.0342	0.0473
B-15	0.144	0.142	0.0462	0.0118	0.0217
B-16	0.0408	0.0430	0.0160	0.0042	0.0021
B-17	0.268	0.218	0.152	0.130	0.166
B-18	0.119	0.210	0.101	0.0754	0.111
B-19	0.0197	0.0197	0.0282	0.0102	0.0166
B-20	0.0132	0.0032	0.0035	0.0019	0.0012

Continued on the next page.

Table 5-3 (continued). CONCENTRATION OF ACIDIC AND NEUTRAL AMINO ACIDS AND AMINO SUGARS IN UNHEATED AND HEATED ALIQUOTS OF THE MIOCENE MALAGA MUDSTONE (micromoles per gram of sediment). The sample numbers are explained in Table III-1 in Appendix III.

Sample Number	Tyrosine	Phenyl-alanine	Glucos-amine	Beta-alanine	Gamma-amino-butyric acid
B-1 (unheated)	*	0.981*	*	0.633	--
B-2	0.0238	*	0.216*	0.297	--
B-3	*	0.345*	*	0.342	--
B-4	0.0122	0.0467	0.0372	0.0403	--
B-5	0.0262	0.0590	0.372	0.456	--
B-6	0.0194	0.0674	0.1010	0.125	0.097
B-7	0.0444	0.067	0.0187	0.0263	0.085
B-8	0.112	0.025	0.0055	0.0316	0.093
B-9	0.0108	0.0176	--	0.0169	0.145
B-10	0.0107	0.0179	--	0.0128	0.336
B-11	0.0096	0.0228	--	0.0118	--
B-12	0.0019	0.0024	--	--	0.083
B-13	0.0224	0.0370	--	0.0341	0.105
B-14	0.0112	0.0208	--	0.0247	0.088
B-15	0.0047	0.0058	--	0.0176	0.155
B-16	0.0009	0.0008	--	0.0079	--
B-17	0.0046	0.0905	0.0555	0.155	--
B-18	0.0186	0.173*	*	0.136	--
B-19	0.0009	0.0038*	*	0.0167	0.105
B-20	0.0017	0.0008	--	0.0035	0.171

The quantity so marked represents the sum of the amino acids marked by an asterisk in the same sample.

VARIATION OF AMINO ACID CONCENTRATIONS WITH TIME OF HEATING

A series of samples of both sediments heated to a maximum temperature in the range of 235° to 245°C are compared. Actually the maximum temperature range for the Malaga mudstone samples is more restricted. It covers the range from 235° to 242°C.

Graphs are provided which illustrate the variation in the yields of the individual amino acids as a function of total heating time. The yields are expressed as percentages of the measured concentration of a given amino acid in the 6 N HCl hydrolyzate of the unheated sediments. The initial concentrations of tyrosine and phenylalanine in the unheated Malaga mudstone samples could not be determined because the peaks did not separate. Therefore, the measured hydrolyzate concentrations are plotted for this sediment. The total heating time for a run is used as the independent variable. The amino acids considered are glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, and beta-alanine. Figures 2 through 11, pages 53 through 62, are the graphs pertaining to the amino acids in the order given above. The data for these figures can be found in Tables 5-2 and 5-3. See pages 42 through 48. See also Table III-2 in Appendix III.

Serine and threonine do not occur in significant amounts in samples heated around 240°C. Although the data are limited, these two amino acids appear to decline regularly with heating time in samples heated between 190° and 200°C. See Tables 5-2 and 5-3.

The sediment samples used in the experiment were well-mixed. The same weight of material was used each time. All of the samples were treated uniformly except for heating time. There were slight variations in the maximum temperature of heating (235° to 245°C) and the thermal histories of the samples. The total heating time is used as the time variable in order to suppress the differences in the early heating histories of the aliquots. Although some erratic data were obtained the results seem to be generally consistent. It is believed that the amino acid yields may be compared directly on the basis of heating time.

Examination of the graphs indicates that there are notable differences in the behavior of the amino acids during heating in the two sediment samples. The curves for the Recent marine sediment resemble, in a gross way, exponential decay curves. This is also true for some of the amino acids in the Miocene sediment. Other graphs for the Miocene sediment which correspond to amino acids known to be rather stable through geologic time show remarkably increased yields at shorter heating times. These amino acids are glycine, alanine, and glutamic acid. It is known that certain amino acids - notably, glycine, alanine, proline, and ornithine - may be produced by the pyrolytic breakdown of other amino acids (Vallentyne, 1964; Degens, personal communication). Glutamic acid is not a pyrolytic product of any other amino acid; yet it shows a remarkable increase in two of the samples and appears to maintain a high level of concentration through the entire series of heating experiments (see Figure 2, page 53).

Glycine, an amino acid which has been shown to develop from several other amino acids during pyrolysis; i.e., threonine, serine, methionine, tyrosine, and valine (Vallentyne, 1964), shows a very rapid decrease in the Recent sediment. Its concentration remains relatively low throughout the experiment. This is probably the result of the high initial concentration of glycine in the unheated sample. The Recent marine sediment would be expected to contain a large amount of the above-mentioned precursors of glycine. Therefore one would expect the curve to decrease more slowly than it does if glycine were being produced in any significant amount at these temperatures. Glycine may also be lost to the sample vial residues. Alanine, another product of the pyrolysis of some amino acids, also continues to remain rather low in the Recent sediment.

Relatively large increases are noted for glutamic acid, glycine, and alanine in some of the Miocene sediment samples heated for shorter periods of time. The increases shown seem to be well outside the limits of error of the measurements. Some of the other amino acids found in the same samples show an apparent parallel increase. These increases are not as marked because the yields do not exceed those measured in the unheated samples.

The fact that the increases take place in as many as three samples with similar thermal histories indicates that the effect is real. The increased yields probably cannot be attributed to contamination since it is highly unlikely that a series of two or three successive runs would be contaminated in such a similar fashion. Bad sampling may be dismissed for the same reason. Also a blank run used

to measure the level of contamination in the experiment was run in the same bomb and furnace and at a similar temperature. The contamination level measured was far below the increase in concentration noted in some of these samples.

It is significant that even after undergoing heating at temperatures above 235°C for a period of more than 40 hours the concentration of some of the amino acids in the Miocene sediment has not been reduced a great deal relative to their initial measured concentrations. This is true for glutamic acid, proline, glycine, alanine, and valine, the most abundant amino acids in the heated sediments. The data obtained from the heated Miocene sediment suggest that short term heating in the presence of water at relatively high pressure increases the availability of amino acids in older rocks to extraction by 6 N HCl. It has not been determined whether amino acids are released by the chemical action of water and other substances present in the sediment sample prior to acid hydrolysis. Similar experiments should be performed with the acid hydrolysis step eliminated.

Threonine and serine show a marked decline with time in the temperature range of 190° to 200°C. It should be pointed out that these amino acids are less stable to acid hydrolysis than the other amino acids found in the sediments. It is possible that the decomposition of these compounds may be aided by surface catalysis. Therefore, their concentrations may be depressed by this step in the procedure. This effect should be checked by heating sediments in the presence of water without subsequent acid treatment. These experiments could not be performed after the original data were acquired.

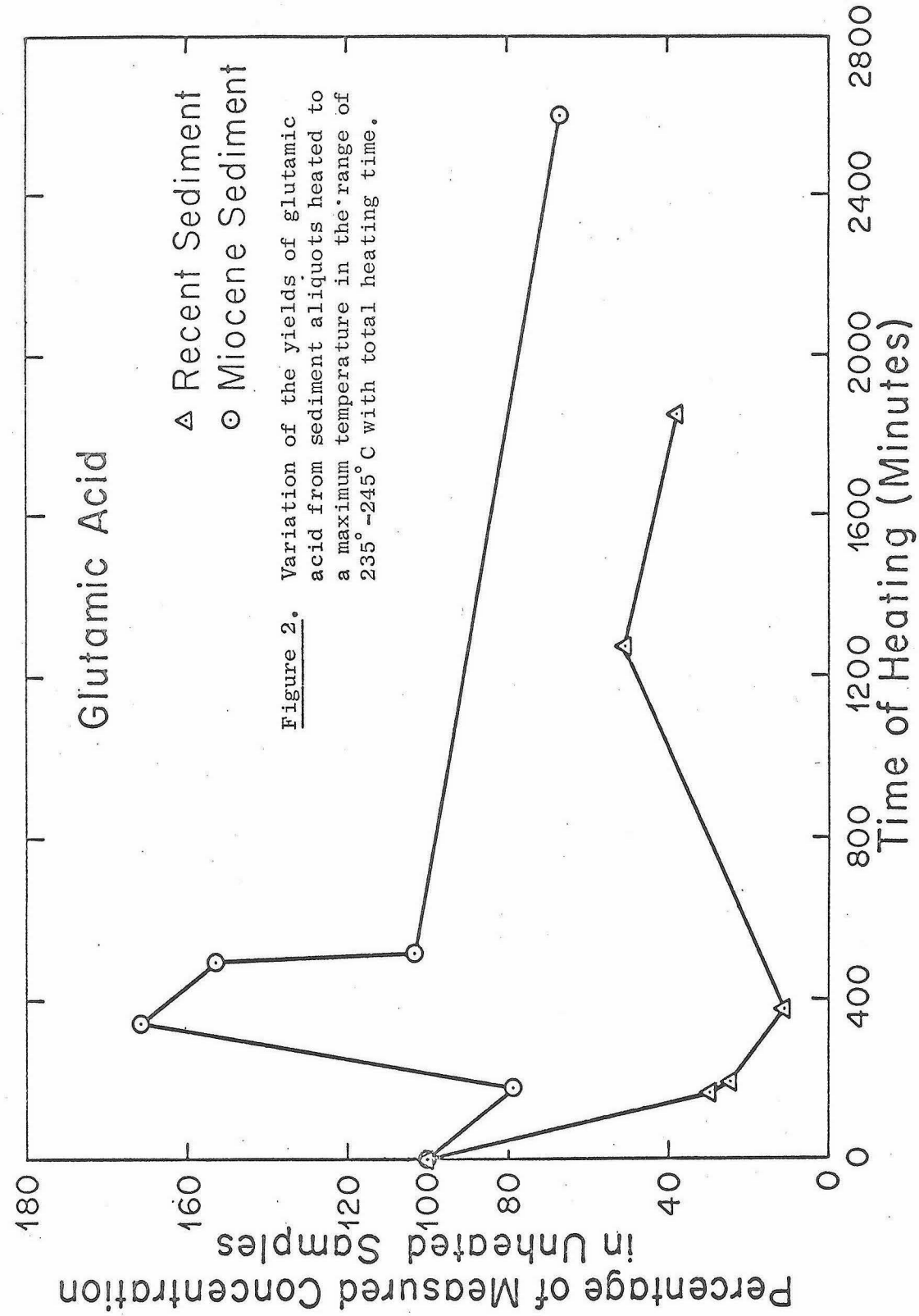

Figure 2. Variation of the yields of glutamic acid from sediment aliquots heated to a maximum temperature in the range of 235°-245°C with total heating time.

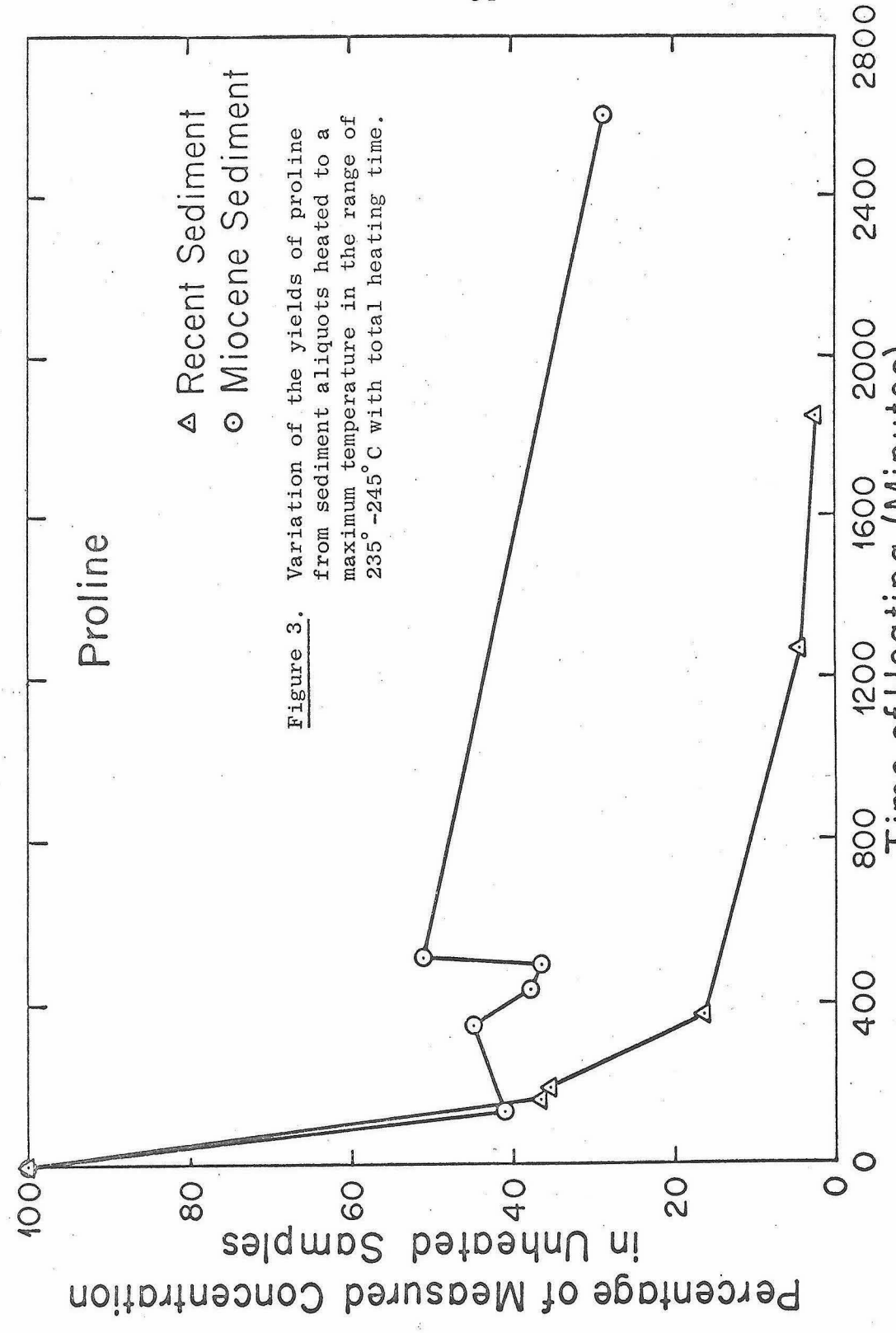

Figure 3. Variation of the yields of proline from sediment aliquots heated to a maximum temperature in the range of 235°-245°C with total heating time.

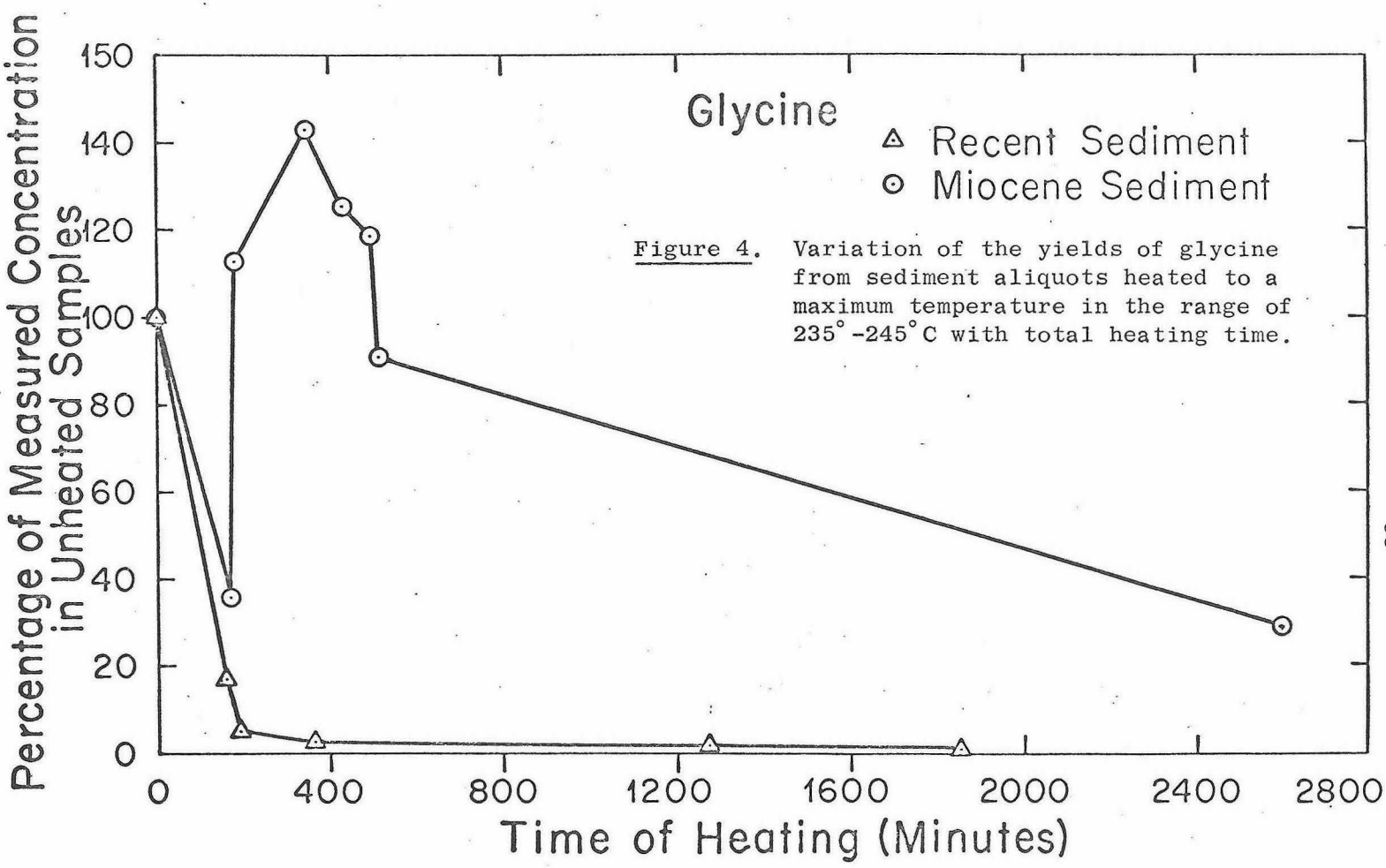

Figure 4. Variation of the yields of glycine from sediment aliquots heated to a maximum temperature in the range of 235°-245°C with total heating time.

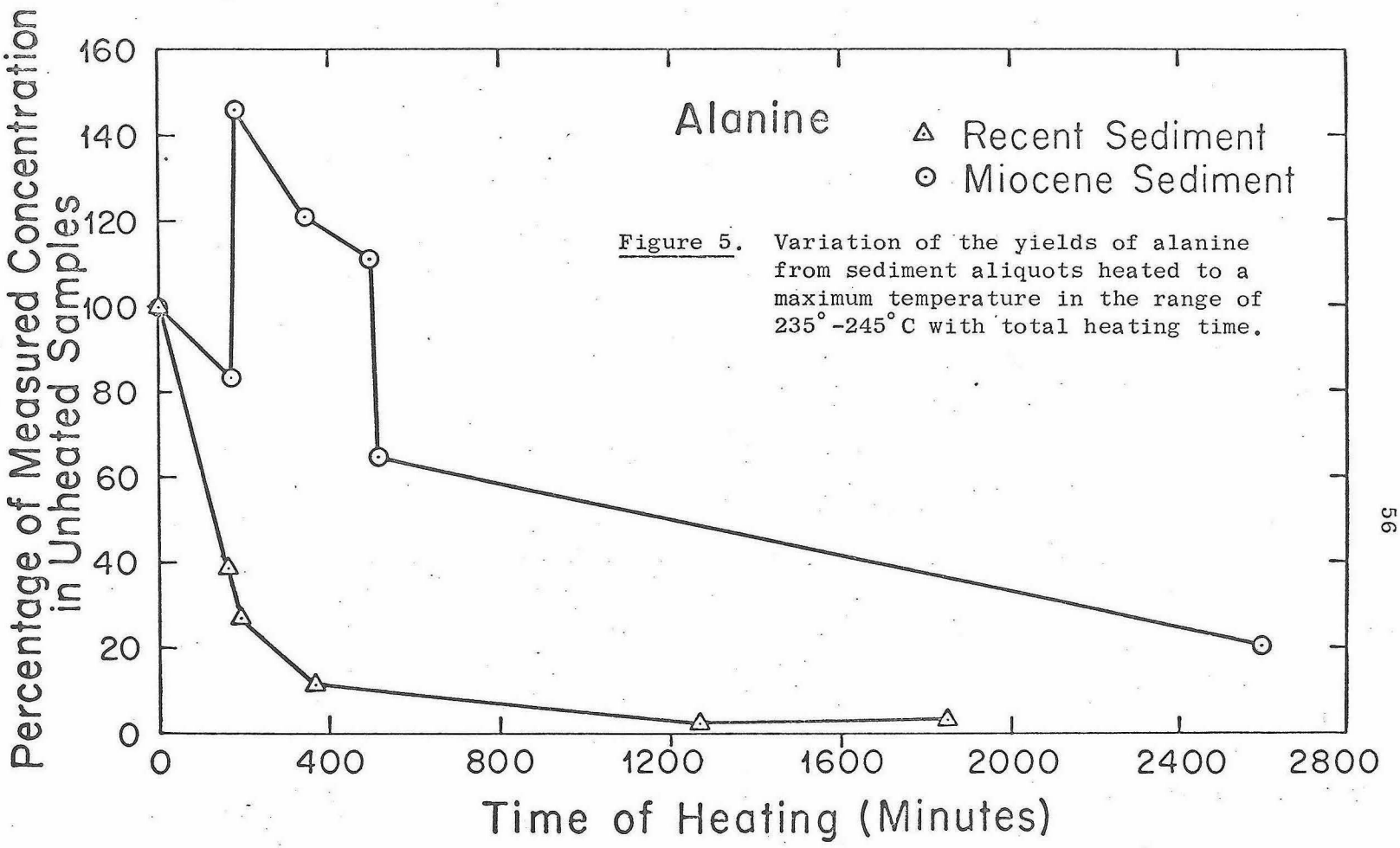

Figure 5. Variation of the yields of alanine from sediment aliquots heated to a maximum temperature in the range of 235°-245°C with total heating time.

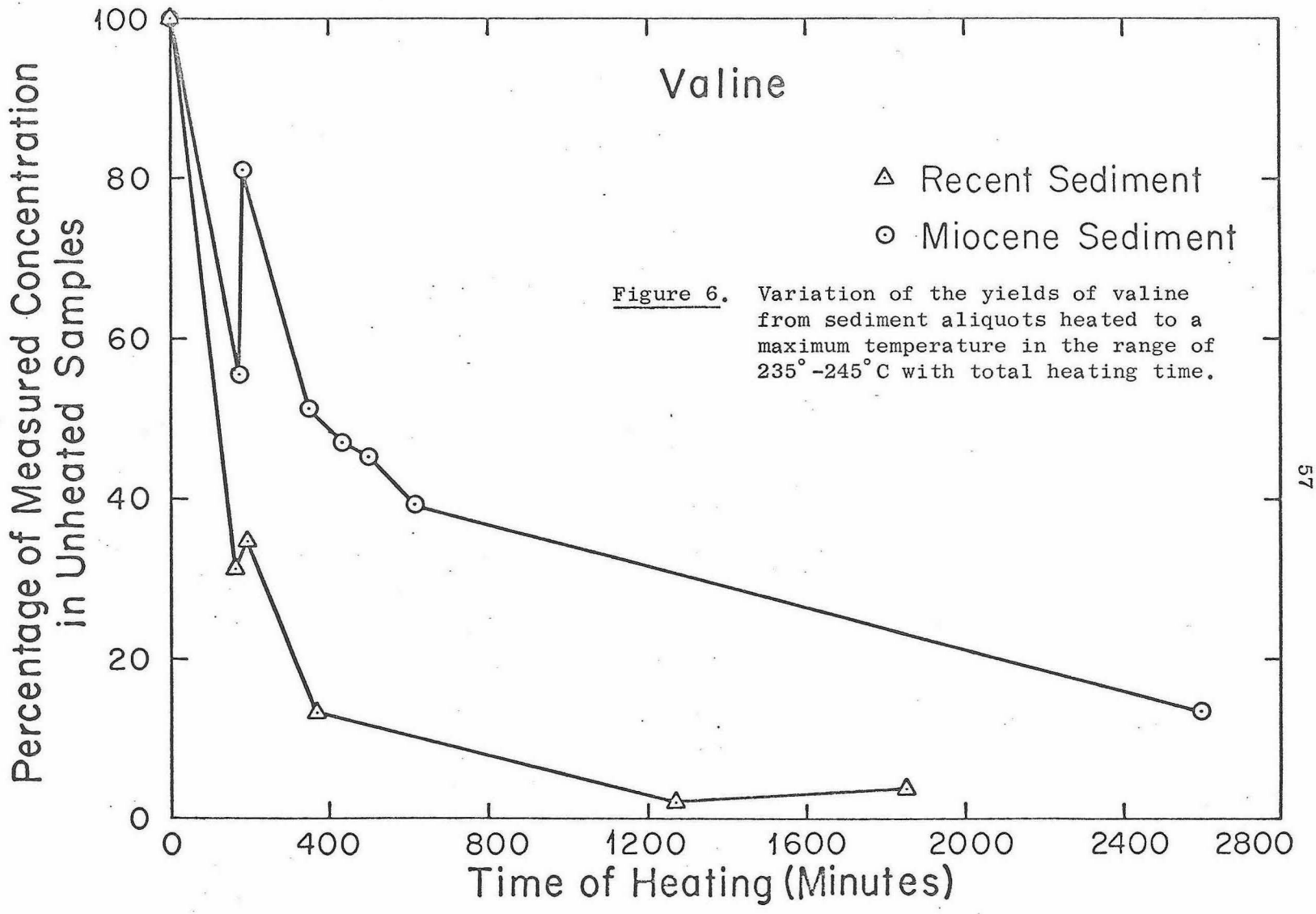

Figure 6. Variation of the yields of valine from sediment aliquots heated to a maximum temperature in the range of 235°-245°C with total heating time.

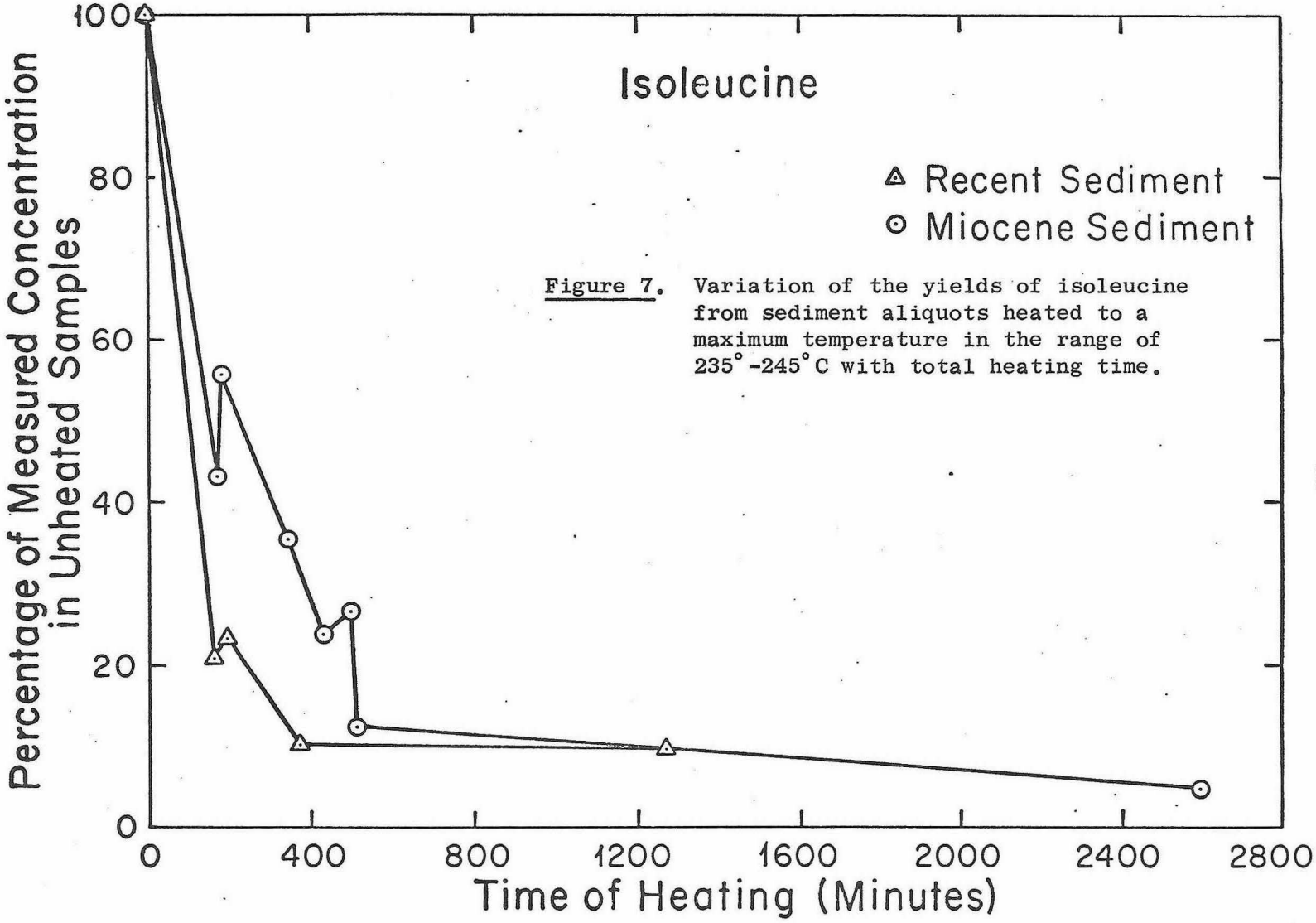

Figure 7. Variation of the yields of isoleucine from sediment aliquots heated to a maximum temperature in the range of 235°-245°C with total heating time.

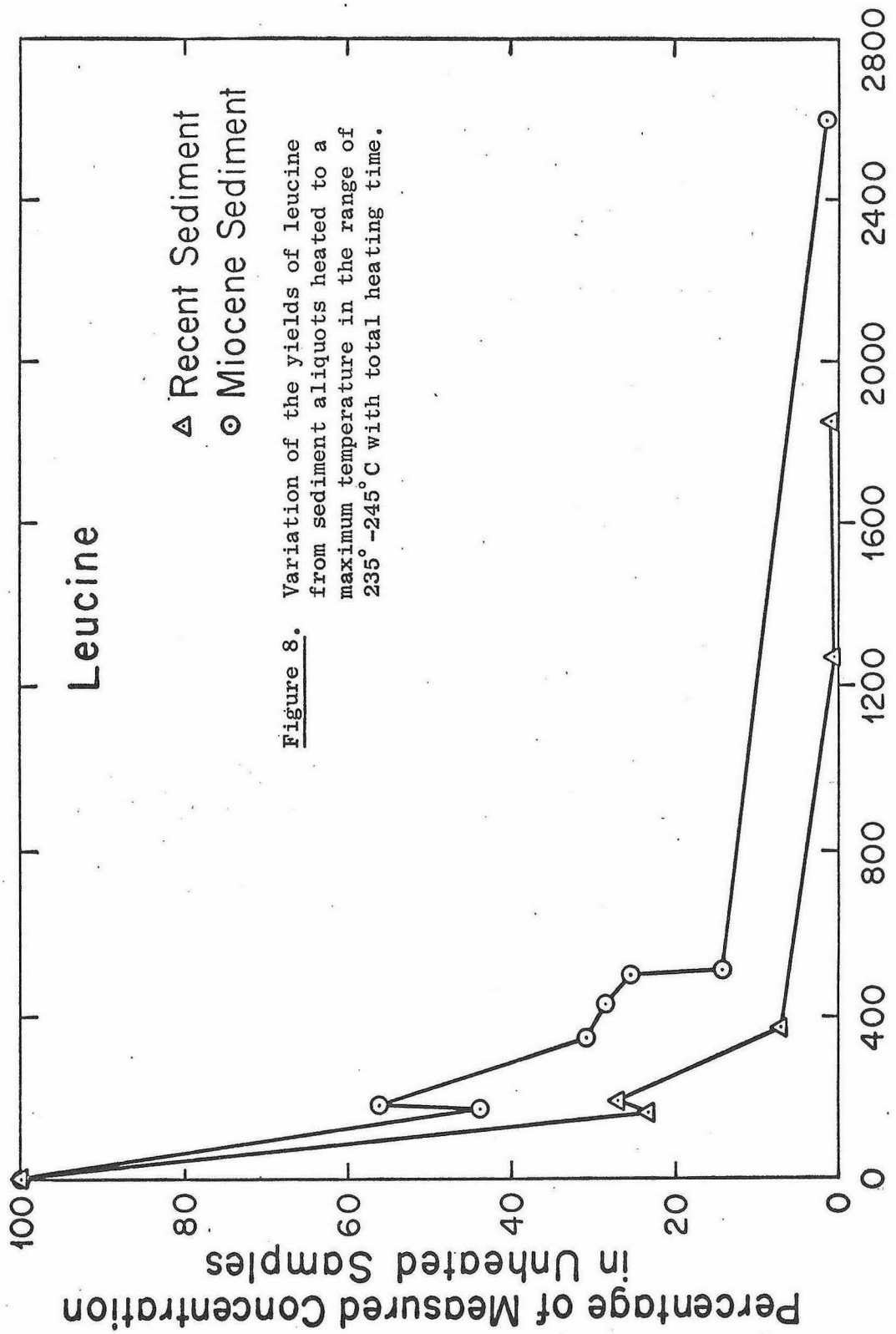

Figure 8. Variation of the yields of leucine from sediment aliquots heated to a maximum temperature in the range of 235°-245°C with total heating time.

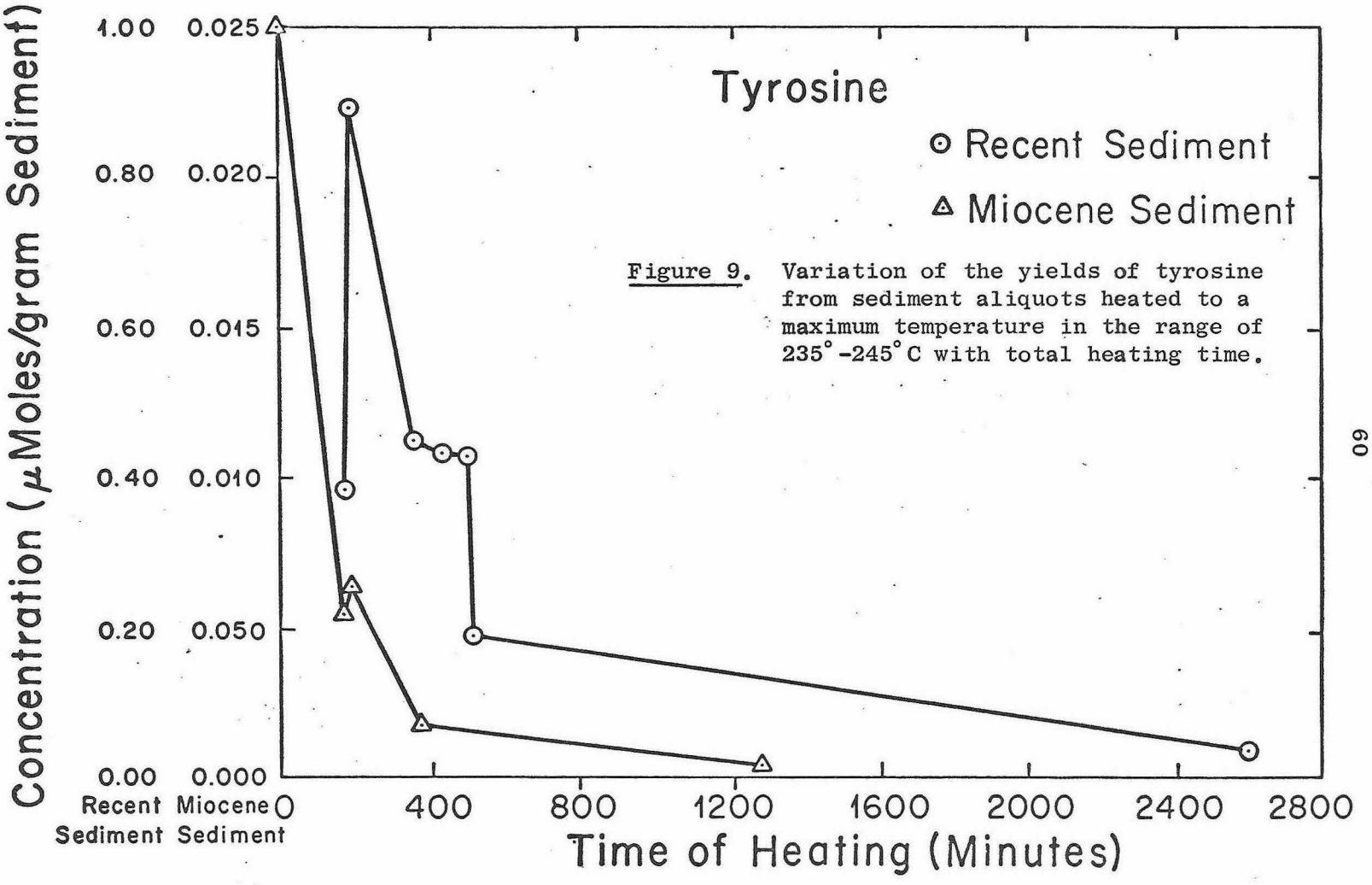

Figure 9. Variation of the yields of tyrosine from sediment aliquots heated to a maximum temperature in the range of 235°-245°C with total heating time.

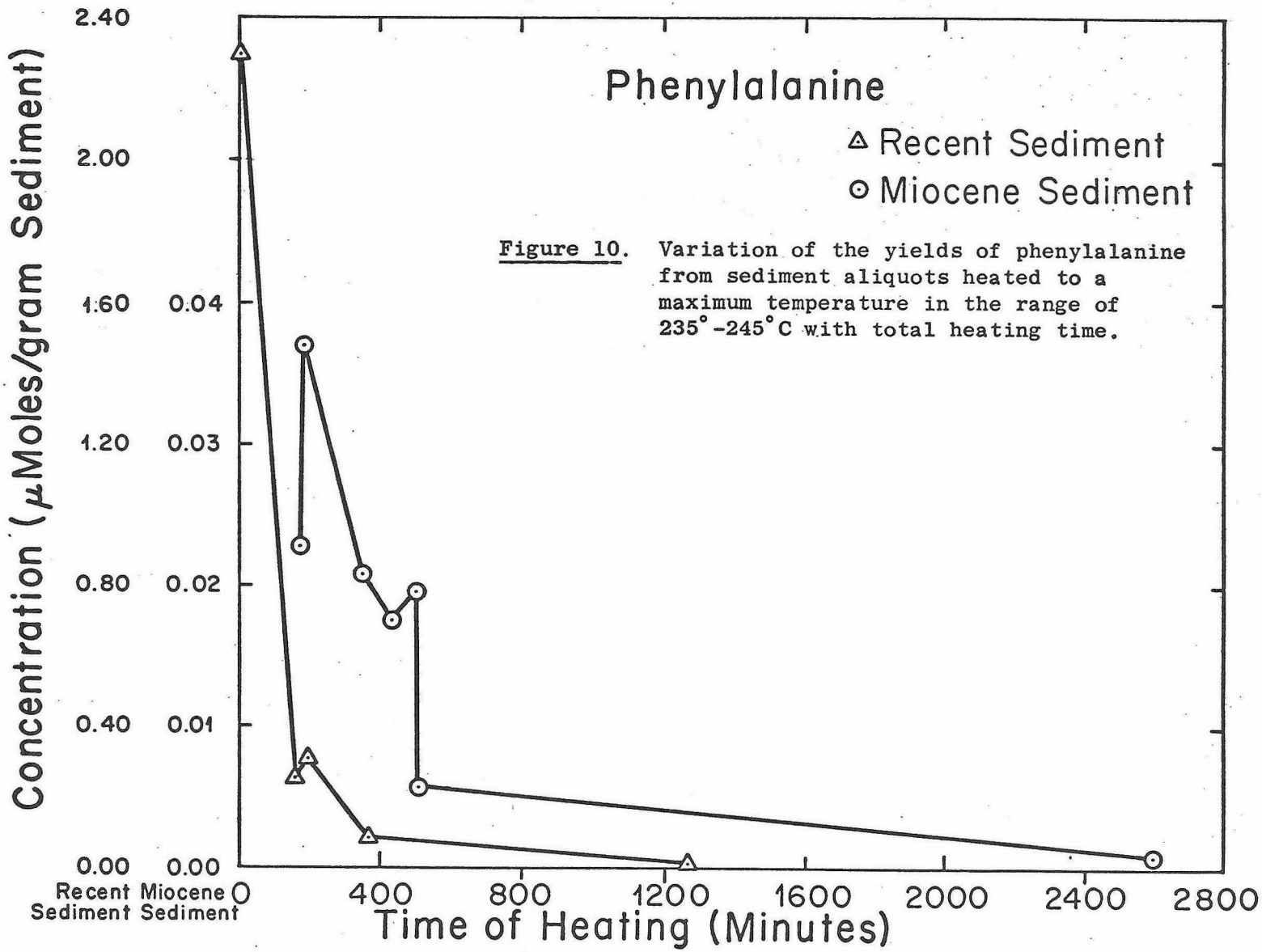

Figure 10. Variation of the yields of phenylalanine from sediment aliquots heated to a maximum temperature in the range of 235°-245°C with total heating time.

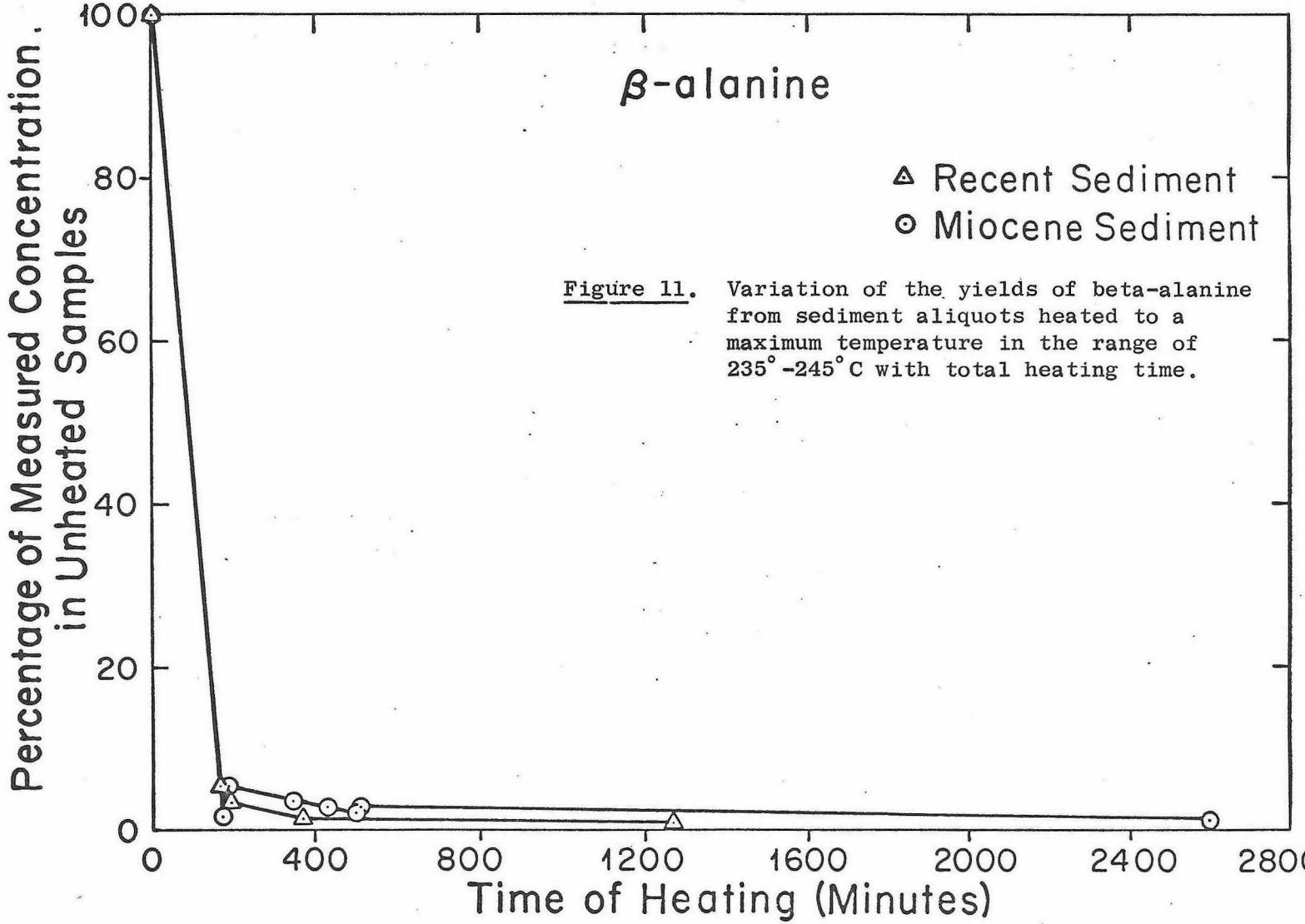

Figure 11. Variation of the yields of beta-alanine from sediment aliquots heated to a maximum temperature in the range of 235°-245°C with total heating time.

RELATIVE STABILITIES OF AMINO ACIDS HEATED IN NATURAL SEDIMENTS

In order to determine the relative thermal stabilities of the amino acids in sediments the yield of each amino acid was normalized to the yield of valine in the same sample. Valine was chosen to normalize the data for the following reasons:

1.) compared to the other amino acids it shows the most regular thermal decomposition behavior in both sediments (see Figure 6, page 57)

2.) it is probably not formed by the thermal decomposition of other amino acids - alanine, which is frequently used for normalization of amino acid data, can be produced by the thermal breakdown of serine and methionine (Vallentyne, 1964)

3.) its peak on the chromatograms is sharper and better separated than those of the other amino acids

4.) it seems to be one of the more stable amino acids.

The yield of valine may be anomalously low in sample A-7, however.

The ratios of the amino acids to valine for the Recent sediment are given in Table 5-4, pages 81, 82, and 83; the data for the Miocene sediment are in Table 5-5, pages 84, 85, and 86. Figures 12, 13, and 14 on pages 78 through 80 show the variation of the ratios with heating time for samples of the two sediments heated in the range of 235° to 245°C. Although the data are limited, the available information is suggestive. The graphs indicate that various amino compounds in sediments respond differently to similar heating conditions. Some of

the amino acids exhibit different behavior in the two sediments.

Because no information is available for the Miocene rock heated for intervals between 513 and 2597 minutes, the data from sample B-16 are considered tentative. It is interesting, however, that many of the values from this sample are fairly consistent with related specimens heated for shorter periods of time.

Recent Marine Sediment

Examination of Figures 12 and 13, pages 78 and 79 respectively shows that glutamic acid and glycine resemble each other in their reaction to heating in the Recent sediment. The yields of these amino acids decrease relative to valine for heating periods ranging up to 370 minutes. Glycine is eliminated faster than glutamic acid. At longer heating intervals these amino acids increase compared to valine. The glutamic acid ratio grows more rapidly than that of glycine. The low yield of valine in sample A-7 may account for part of the large relative increase of glutamic acid and glycine in this specimen.

The complex behavior of the glutamic acid and glycine ratios in the Recent sediment may be explained in the following way. In surface sediments from nearshore basins the amino acids are probably present in two major reservoirs. They are:

1.) proteins and peptides from living and recently dead organisms (predominantly microorganisms)

2.) amino acids structurally held in complex sedimentary organic matter, which may be a combination of land-derived material (soil organic matter and macerated

plant remains) and heteropolycondensates formed authigenically in the sediments during diagenesis. The amino acids in the proteinaceous material are probably more mobile than those in humic complexes since most proteins, peptides, and free amino acids are appreciably soluble under neutral pH conditions. Structural proteins have rather low solubilities, however. Dissolved constituents are capable of moving freely in the water present in the bomb. They may make frequent contact with the surfaces of mineral grains and the bomb. Thermal decomposition of amino compounds is probably affected by surface catalysis (Hunt, personal communication). Thus a major portion of the acid-soluble amino compounds in the Recent sediment may decompose faster than the same substances in pure aqueous solution. The decline of glutamic acid and glycine relative to valine indicates that they may be more easily degraded than the latter in the presence of a catalyst.

Partial disruption of an organic condensate by the action of H_2O at high temperature and pressure may make the amino acids structurally held in the complex more available to acid hydrolysis. The data from the Miocene rock and the humic acid extracts used in this study indicate that such a process may occur. In general, glutamic acid and glycine are more abundant than valine in the humic acid extract from the Recent sediment. The higher ratios in the Recent sediment aliquots, heated for longer periods of time, probably reflect the relative stabilities of amino acids in the humic complex. Amino acids fixed in an organic complex would not be affected by surface catalysis as much as soluble amino compounds.

Some of the low values for glycine may result from the formation of residues in the sample vials. These vial residues contain glycine in much greater quantity than any of the other amino acids.

According to Vallentyne (1964) glycine may be derived by the thermal degradation of several amino acids; i.e., threonine, serine, methionine, tyrosine, and valine. Glutamic acid does not arise from the pyrolysis of any known amino compound. Glycine does not appear to be formed at temperatures around 240°C in the Recent sediment. The rapid decrease of the proportion of glycine to valine indicates this. However, in the aliquots heated at lower temperatures glycine may be formed from other amino acids.

It is not possible to evaluate the interconversion of amino acids from the data of this experiment. Only ninhydrin-positive compounds were measured. Furthermore, it is unlikely that the actual concentrations of the amino acids in the sediments were measured. Therefore, no material balance calculations were attempted.

The ratio of proline to valine in unheated and heated aliquots of the Recent sediment are generally similar. This is also true for alanine although the data are more scattered. The thermal stabilities of proline and alanine are probably like that of valine in the Recent sediment when heated in a metal bomb at temperatures around 240°C. At lower temperatures the proline ratio remains fairly similar to that in the unheated sediment. Alanine shows a relative increase with heating time in the same aliquots.

Experiments have shown that proline may be produced by the pyrolysis of arginine (Degens, personal communication; Vallentyne,

1964). This reaction may be catalyzed by clay minerals (Degens, personal communication). Alanine also forms from the thermal degradation of serine and methionine (Vallentyne, 1964). In general, proline and alanine are only found in trace amounts when produced by pyrolysis in pure aqueous solutions.

The slightly increased ratios for proline and alanine may reflect the growth of these compounds from the amino acids mentioned above. This cannot be determined from the available data. Proline and alanine are more abundant than valine in the humic acids extracted from this sediment.

The ratio of tyrosine to valine decreases very gradually with heating time at temperatures around 240°C in the Recent sediment. See Figure 14 and Table 5-4. The initial value is rather low (0.41). The tyrosine ratios obtained from the heated specimens of this material fall within a very narrow range (0.21 to 0.30). Tyrosine in the more labile organic fraction of the sediment appears to be slightly less stable than valine. The proportions of tyrosine to valine in heated and unheated aliquots of the Recent humic acid are very close to 0.3. The source of the tyrosine and valine measured in the samples heated for longer periods may be the humic acid fraction of this sediment. When hydrolyzed with HCl, amino acids are probably found in proportion to their abundance or availability in the organic complex unless they are unstable in an acidic medium.

Phenylalanine decreases with respect to valine in this sediment when heated for various periods of time in the neighborhood of 240°C. See Figure 14. Phenylalanine seems to be more

labile than valine in this sediment. The decline of phenylalanine relative to valine is rather abrupt for the samples heated less than 370 minutes. The curve appears to flatten out at longer heating periods. The rapid decrease of the ratio indicates that phenylalanine is much less stable than valine in the more labile portion of the sediment.

The proportion of leucine to valine diminishes in the Recent sediment with heating time. The data for the specimens heated around 240°C are plotted in Figure 14. The initial ratios for leucine and phenylalanine in the unheated Recent sediment are identical. The decline of the leucine ratio is not as abrupt as that for phenylalanine. This indicates that leucine is probably more stable than phenylalanine in the same samples. The stability of leucine is similar in the two sediments used in this experiment.

Isoleucine decreases relative to valine in samples of the Recent sediment heated in the range of 235° to 245°C; the decline appears very gradual. The isoleucine ratio for sample A-7 seems excessively high. Since leucine and isoleucine are almost alike structurally one would expect them to react to heating in a similar way. The heating curves for these two amino acids in the Recent sediment resemble each other slightly. Although the initial ratios differ considerably in the two sediments, the data for isoleucine and leucine show a similar trend in both materials.

The proportion of beta-alanine to valine decreases rapidly with heating at all temperatures. Beta-alanine appears to be much less stable than valine in the Recent sediment.

Aspartic acid, threonine, and serine are very unstable relative to valine in the Recent sediment. The results of laboratory experiments on pure aqueous solutions of these three amino acids have shown that they are rather unstable thermally.

Gamma-aminobutyric acid is found in HCl hydrolyzates of pyrolyzed glutamic acid solutions (Povoledo and Vallentyne, 1964). It is apparently formed by the decarboxylation of pyrogultamic acid, the lactam of glutamic acid, and subsequent opening of the pyrrolidone ring with HCl.

Because of the relationship of gamma-aminobutyric acid to glutamic acid the sums of the yields of the two compounds are compared to those of valine. The data obtained from the sediment samples heated around 240°C are plotted in Figure 13.

The hydrolyzate of the unheated Recent sediment contained an appreciable amount of gamma-aminobutyric acid. This is not surprising since gamma-aminobutyric acid is important in the metabolism of green plants (Fruton and Simmonds, 1958). The ratios of the sum of glutamic and gamma-aminobutyric acids increase markedly with time during heating. This indicates that the combined acids are much more stable than valine. These two amino acids, individually, are found in larger absolute amounts in some samples heated for longer periods. The insoluble humic substances in the sediments are altered by the action of H_2O at high temperature and pressure; this probably permits the process of HCl hydrolysis to release larger quantities of amino acids. Therefore, the rapid increase of the ratio with heating time is probably the result of the following two factors working together:

1.) the high thermal stabilities of glutamic and gamma-aminobutyric acids

2.) the relatively large concentrations of these amino acids in the humic substances of the sediment.

It is possible that the glutamic acid form is more abundant than the lactam form in the humic fraction of the sediment since this molecule has three very reactive substituent groups exposed. Glutamic acid may, therefore, serve to cross-link the humic acid polymer.

Summary

According to the data from aliquots of the Recent sediment heated in the neighborhood of 240°C, glutamic acid, glycine, and phenylalanine have thermal stabilities which vary with heating time as well as temperature. This suggests that there are at least two major amino acid reservoirs in the sediment. Proline and alanine resemble valine in the same heated samples. Although the data are sparse and widely spaced, it appears possible that the ratios of proline and alanine increase with heating time. Tyrosine, isoleucine, and leucine decrease gradually relative to valine. Beta-alanine, threonine, serine and aspartic acid are much less stable than valine.

Most of the amino acids found in the hydrolyzates of the Recent sediment probably were released from proteinaceous material during the experiment. The relative thermal stabilities of the amino acids in this fraction of the sediment differ slightly from those obtained by heating the individual acids in pure aqueous solution. The amino acids have been grouped according to their thermal stabilities,

relative to valine, in samples of the Recent sediment heated around 240° C. The groups, arranged in order of decreasing stability, are: (1) proline, alanine, valine; (2) glutamic acid, tyrosine, isoleucine, leucine; (3) phenylalanine, glycine; (4) beta-alanine, threonine, serine, aspartic acid. Gamma-aminobutyric acid may also belong in group (1). Surface catalysis and the state of combination may affect the relative rates of decomposition. The sequence is based upon a comparison of the rates of decline of the amino acids in the Recent sediment aliquots heated for short periods of time.

The rapid rise of the glutamic acid, gamma-aminobutyric acid, and glycine ratios with continued heating suggests that a second, more stable, source of amino acids exists in this sediment. After a sudden decline, the curve for the phenylalanine ratio tends toward a constant value in the aliquots of the Recent sediment heated for longer intervals. If the behavior of the amino compounds in samples of this sediment heated for longer periods is considered separately, then a slightly different sequence of relative thermal stabilities is obtained. The amino acids may be grouped in the following way: (1) glutamic acid, glycine; (2) proline, alanine, valine, tyrosine, phenylalanine; (3) isoleucine, leucine; (4) beta-alanine, threonine, serine, aspartic acid. These amino acids behave very much like their counterparts in the Miocene rock.

Glutamic acid and glycine are more abundant than valine in the humic acid fraction of the Recent sediment. The ratios of tyrosine to valine found in the heated samples of this sediment are very similar to that in the Recent humic acid extract. The concentrations of the

amino acids in the humic complex may influence the relative thermal stabilities inferred from the heating experiments since the samples were hydrolyzed with HCl after they were heated. Nevertheless, the similarity between the sequences in the two sediments is significant considering the great difference in the ages of the materials. The humic acid fractions of both sediments are compared in a later section of this work.

Miocene Malaga Mudstone

This experiment has shown that several aliquots of this sediment provide larger amounts of some amino acids than the unheated rock. These results do not agree with our understanding of the thermal stabilities of amino acids in pure aqueous solution. It can be seen that greater quantities of amino compounds become available to acid hydrolysis after the rock is subjected to the action of H_2O at high temperature and pressure. Those amino acids found in greater amounts after heating are generally the most abundant ninhydrin-positive substances in the unheated sediment. They appear to be among the more common amino compounds in living tissue and are known to be rather stable. It is not surprising that the most frequently found amino acids in living organisms are also very stable.

The ratios of the individual amino acids from samples of this sediment heated around 240°C are compared with their counterparts from the Recent sediment in Figures 12, 13, and 14. If amino acids were released from a statistically uniform source without being altered in any way, then their proportions to valine would remain essentially

constant. The ratios for an amino compound with thermal degradation characteristics very similar to valine would also fall on a horizontal line. Substances with thermal stabilities greater than valine would provide ratios which enlarge with heating time; diminishing ratios would be obtained from compounds which are more labile than valine. Amino compounds which are abundant in an organic complex and are also thermally unstable would be expected to decrease rapidly after heating. Acid hydrolysis of that part of the humic fraction which remains essentially intact during the experiment may liberate amino acids in quantities proportional to their concentration in the complex.

The graphs for alanine, glycine, glutamic acid, and the sum of glutamic and gamma-aminobutyric acids show that these amino acids increase relative to valine when heated for intervals up to 513 minutes long. It can be seen that the values for glutamic acid in samples B-8, B-9, and B-12 are very low. These samples were heated 180, 427, and 360 minutes respectively. The ratios of the sum of glutamic and gamma-aminobutyric acids from these specimens line up very well with the other data. See Figure 13. The regularity of these ratios may indicate that the parent-daughter relationship between the two amino acids is effective in the heated sediments. The ratio increases very rapidly with heating time. Although a straight line has been fitted to the data it is possible that the increase is parabolic. It is interesting to note that the small segment of the curve for the Recent sediment almost parallels that of the Miocene rock.

The data for alanine, glycine, and glutamic acid scatter considerably. However, they definitely indicate an increase in the

ratios for heating periods less than 513 minutes. The actual shapes of the curves cannot be determined. The ratios for glycine and alanine from aliquots heated at lower temperatures also are generally greater than those from the unheated Miocene rock. It is concluded that alanine, glycine, glutamic acid, and gamma-aminobutyric acid are more stable than valine in the Miocene sediment when heated in a bomb with H_2O.

The proportions of proline to valine remain essentially constant in both unheated and heated specimens of the Miocene sediment. See Figure 13 and Table 5-4. Proline and valine seem to have similar thermal stabilities in this rock.

The values for isoleucine and leucine diminish with heating time in the vicinity of 240°C. The ratios for both amino acids decrease in a very similar fashion although it is possible that isoleucine is a bit more stable than leucine. The rate of decline in the Miocene rock seems to be like that in the Recent sediment. These two amino acids will be discussed further in a later section. Both are less stable than valine in the Miocene sediment.

No ratios are available for tyrosine and phenylalanine in the unheated Miocene rock. The values for both amino acids in the samples heated around 240°C appear, for the most part, to be constant. Therefore, it is difficult to determine whether any changes take place in the ratios with heating time. The specimens heated at lower temperatures do not aid in determining how the concentrations of these amino acids are changing relative to valine. The variation of the tyrosine and phenylalanine ratios in these samples is not very consistent.

Beta-alanine, which is abundant in the unheated Miocene rock, does not appear to be very stable in this sediment around 240°C. This is also true for the Recent sediment. The proportion of beta-alanine to valine falls very abruptly after heating. See Table 5-4. However, the beta-alanine ratio remains rather constant after the sudden initial decrease. The proportion of beta-alanine to valine in the more severely treated samples of the Miocene humic acid extract are also rather alike. Lower temperatures were used for heating the humic acids. The beta-alanine ratio in the unheated Miocene humic acid extract resembles the initial value in the bulk sediment. A large portion of the beta-alanine content of the sediment is apparently destroyed during heating. This amino acid is probably released by acid hydrolysis in rather constant proportions to valine from the organic complex not completely altered during heating.

Threonine and serine are reduced to very low levels in the sediment aliquots heated around 240°C. Serine is less abundant than threonine in the unheated Miocene rock. The yield of serine declines less rapidly than that of threonine in the heated Miocene sediment. Serine is apparently more resistant to thermal degradation than threonine in this rock. Both are obviously less stable than valine.

Aspartic acid is found in very low concentrations in the hydrolyzates of the unheated and heated Miocene sediment aliquots. Therefore, not much can be said about its relative thermal stability in this rock.

Summary

Alanine, glutamic acid, glycine, and gamma-aminobutyric acid increase relative to valine in the Miocene rock after short periods of heating around 240°C. The proportion of proline to valine shows little change in the same temperature region. Tyrosine and phenylalanine yield ratios which have narrow ranges indicating that these amino acids may have stabilities similar to valine. No data are available for tyrosine and phenylalanine in the unheated sediment. Therefore, such a conclusion must be considered tentative. Isoleucine and leucine decline compared to valine. Beta-alanine decreases rapidly after heating. The beta-alanine ratios in the samples heated around 240°C are very similar. This may indicate that beta-alanine and valine are released after heating, by acid hydrolysis, in proportion to their concentrations in the undegraded part of the sedimentary organic complex. Serine and threonine are much less stable than valine in this rock. They appear to be more stable than in the Recent sediment, however. Serine is apparently more stable than threonine in this sediment. Aspartic acid is found in low concentrations in both the unheated and heated Miocene samples; therefore, it is not possible to determine its relative thermal stability. Small amounts of aspartic acid are probably released by acid hydrolysis from the undegraded humic complex of the heated samples. Aspartic acid is found in the thermally altered Miocene humic acid extract.

The amino acids have been grouped according to their relative thermal stabilities in the Miocene Malaga mudstone at temperatures

around 240° C. The groups, in order of decreasing relative stability, are: (1) alanine, glycine, glutamic acid; (2) proline, valine, tyrosine (?), phenylalanine (?); (3) isoleucine, leucine; (4) beta-alanine, serine, threonine, aspartic acid (?). Gamma-aminobutyric acid may belong in group (1).

The relative thermal stability sequences of the amino acids in both sediments are almost identical, if only the samples of the Recent sediment heated for greater than 370 minutes are considered. This has been discussed in connection with the Recent sediment. The relative thermal stabilities of some of the amino compounds in the Recent sediment heated for shorter periods differ from those in the Miocene rock. This is believed to result from differences in the structural chemistry of the sedimentary organic substances which contain the amino acids.

The amino acids in the labile organic fraction (proteinaceous material) are probably held together by peptide bonds. How the amino compounds are combined in the humic complex is unknown. The humic materials may be highly cross-linked polymers. There is evidence that the decomposition of alanine in the more labile organic fraction of the Recent sediment is catalyzed during the experiments. In this sediment the activation energy calculated for the thermal degradation of alanine is considerably lower than that determined for this amino acid in pure aqueous solution.

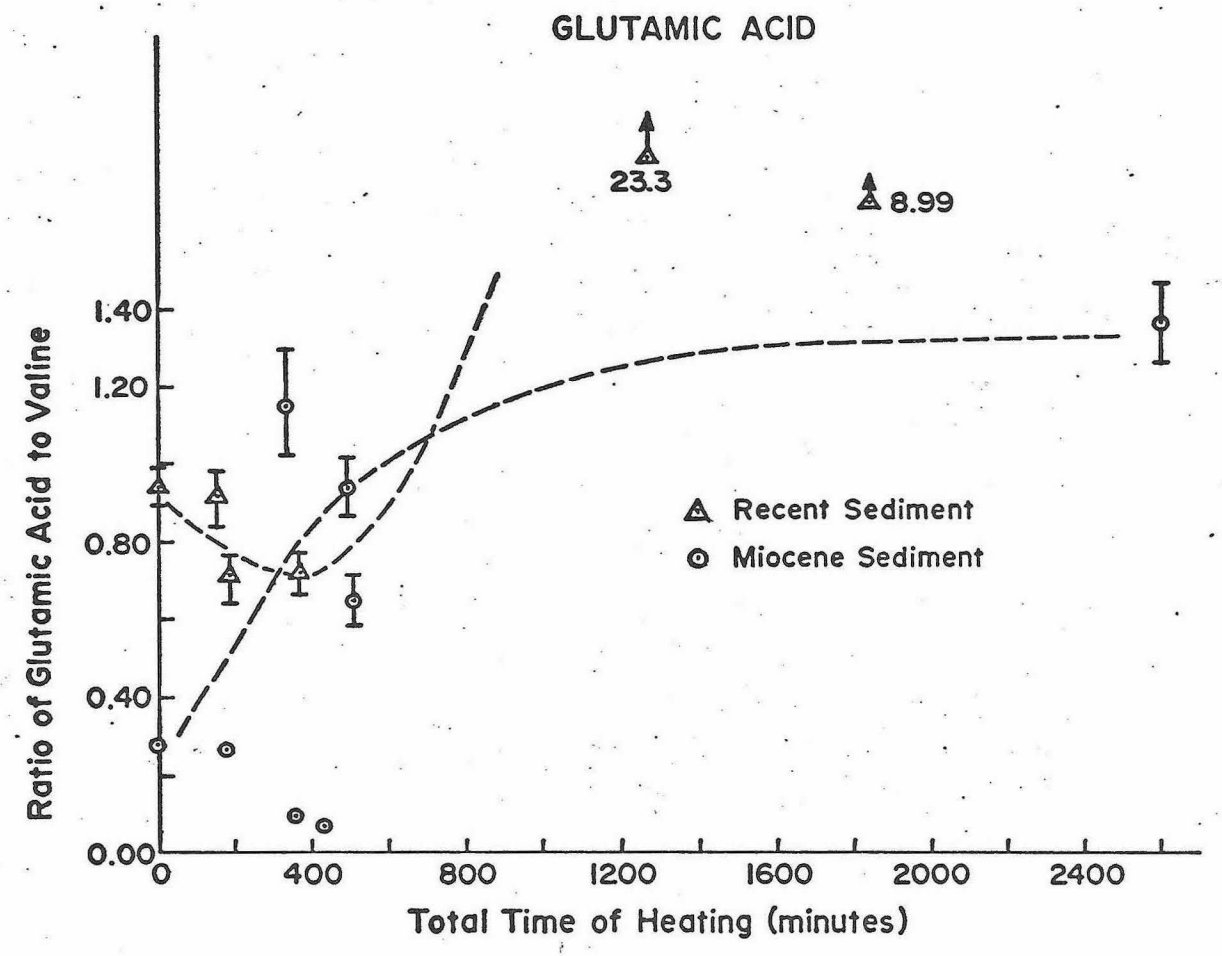

Figure 12. Variation with total heating time of the ratio of glutamic acid to valine for aliquots of Recent and Miocene marine sediments heated to a maximum temperature in the range of 235°-245°C.

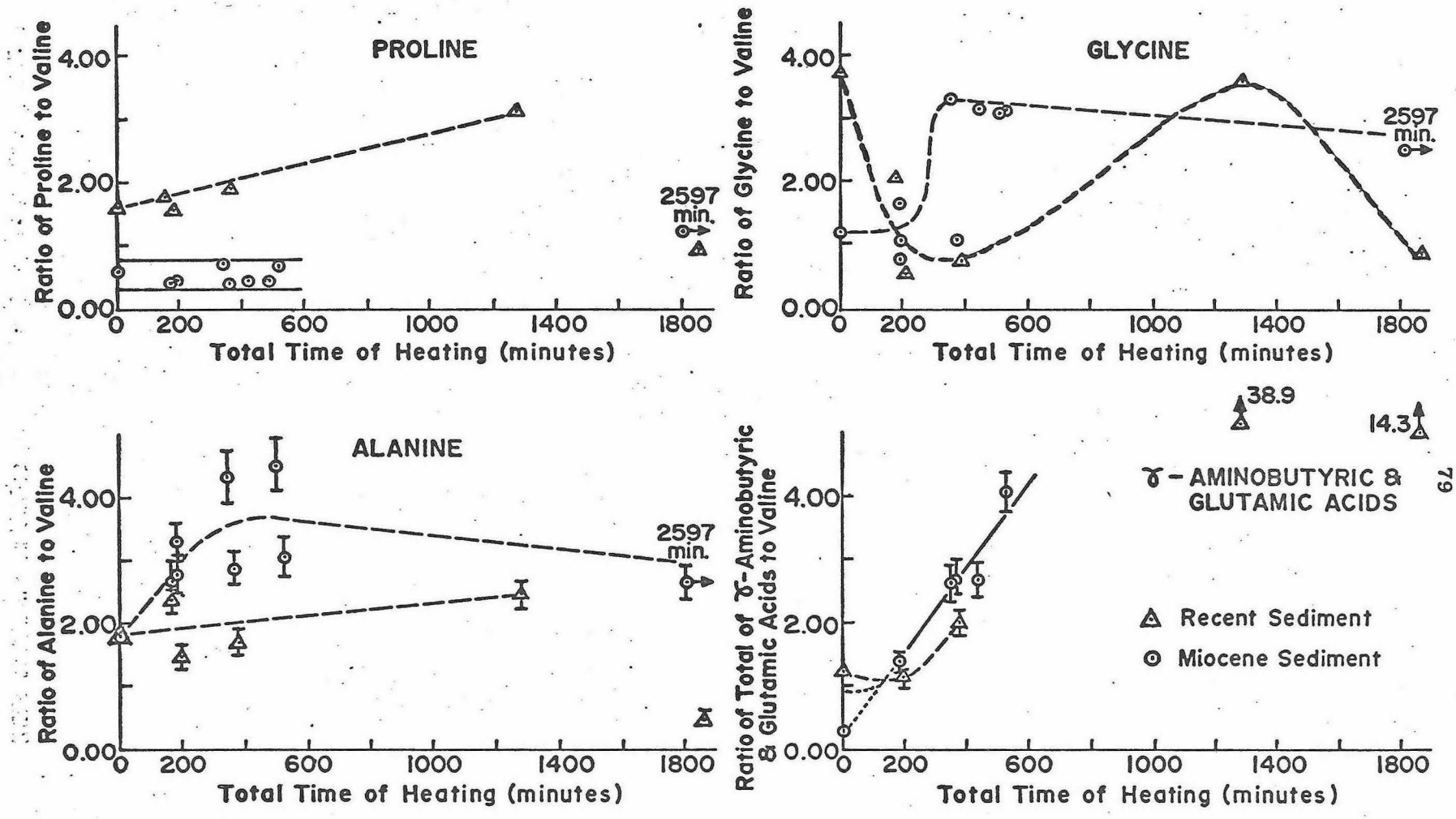

Figure 13. Variation with total heating time of the ratios of proline, glycine, alanine and the sum of glutamic and gamma-aminobutyric acids to valine for aliquots of Recent and Miocene sediments heated to a maximum temperature in the range of 235°-245°C.

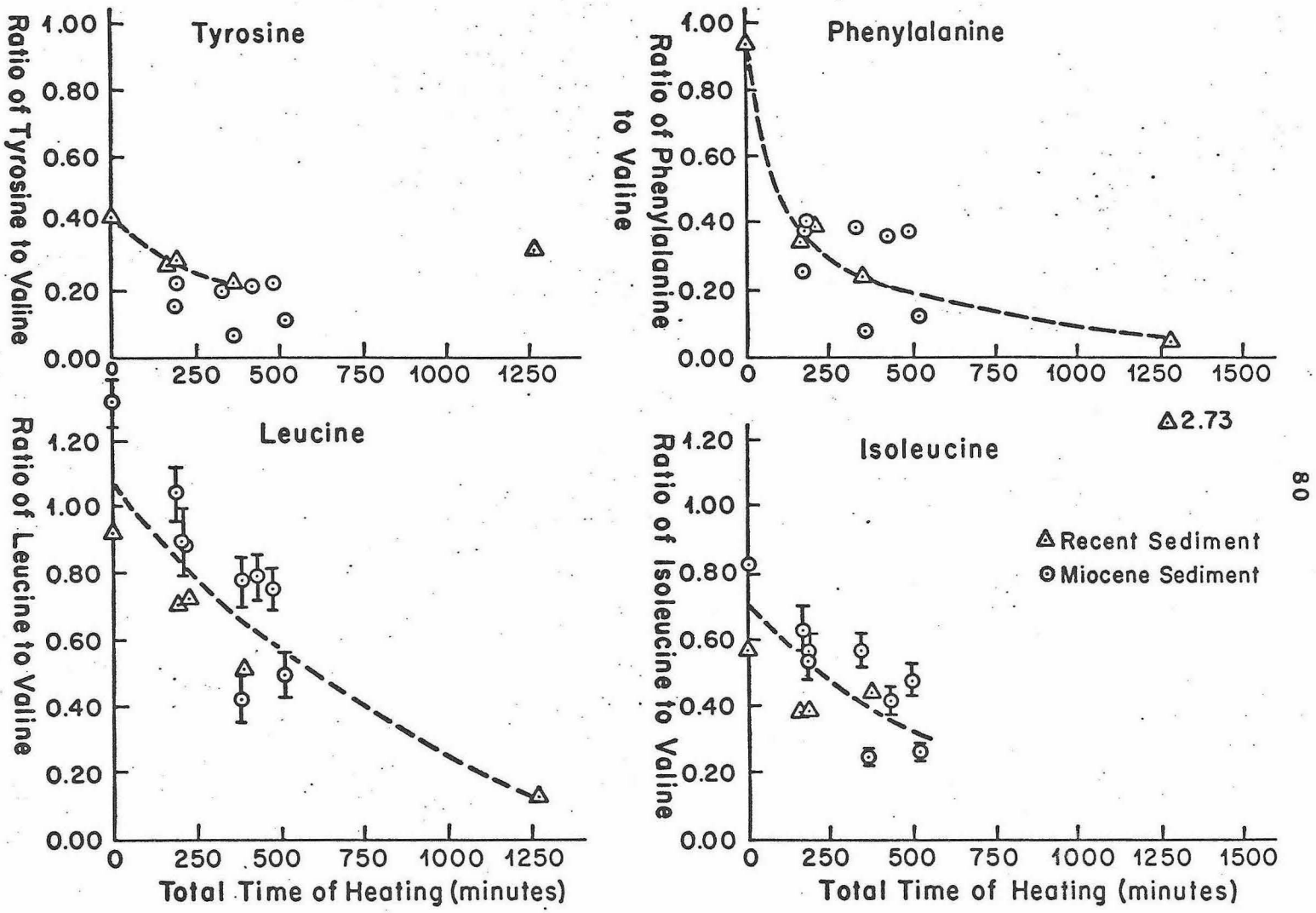

Figure 14. Variation with total heating time of the ratios of tyrosine, phenylalanine, leucine, and isoleucine to valine for aliquots of Recent and Miocene sediments heated to a maximum temperature in the range of 235°-245°C.

Table 5-4. RATIOS OF INDIVIDUAL AMINO ACIDS TO VALINE IN ALIQUOTS OF A RECENT MARINE SEDIMENT HEATED AT VARIOUS TEMPERATURES.

Sample Number	Time (Min.)	Aspartic Acid	Threonine	Serine	Glutamic Acid	Proline
A-1	(unheated)	0.69	0.92	1.17	0.94	1.52
Temperature range 157°-162°C						
A-2	88	0.47	1.00	0.94	0.79	1.27
A-3	354	0.34	0.84	0.81	0.64	1.39
Temperature range 190°-200°C						
A-4	120	0.11	0.93	0.55	0.45	1.35
A-13	240	0.03	0.32	0.12	0.26	1.55
A-5	479	0.03	0.07	0.05	0.35	1.41
Temperature range 235°-245°C						
A-8	161	--	--	--	0.91	1.77
A-6	193	0.10	--	--	0.70	1.54
A-9	367	--	--	--	0.79	1.88
A-7	1269	0.17	--	--	23.3	3.04
A-10	1850	--	--	--	8.99	0.88

Continued on next page.

Table 5-4 (continued). RATIOS OF INDIVIDUAL AMINO ACIDS TO VALINE IN ALIQUOTS OF A RECENT MARINE SEDIMENT HEATED AT VARIOUS TEMPERATURES.

Sample Number	Time (Min.)	Glycine	Alanine	Iso-leucine	Leucine
A-1	(unheated)	3.70	1.86	0.57	0.93
Temperature range 157°-162°C					
A-2	88	2.10	1.64	0.58	0.83
A-3	354	3.64	2.18	0.58	0.83
Temperature range 190°-200°C					
A-4	120	3.09	1.70	0.64	0.79
A-13	240	3.15	1.59	0.54	0.84
A-5	479	1.93	2.28	0.36	0.62
Temperature range 235°-245°C					
A-8	161	2.05	2.32	0.39	0.70
A-6	193	0.57	1.45	0.39	0.72
A-9	367	0.73	1.66	0.44	0.49
A-7	1269	3.60	2.41	2.73	0.11
A-10	1850	0.82	0.44	--	0.19

Continued on next page.

Table 5-4 (continued). RATIOS OF INDIVIDUAL AMINO ACIDS TO VALINE IN ALIQUOTS OF A RECENT MARINE SEDIMENT HEATED AT VARIOUS TEMPERATURES.

Sample Number	Time (Min.)	Tyrosine	Phenyl-alanine	Beta-alanine	Glutamic Acid + Gamma-Amino-butyric Acid
A-1	(unheated)	0.41	0.94	1.21	1.16
Temperature range 157°-162°C					
A-2	88	0.25	0.49	0.56	1.14
A-3	354	0.25	0.59	0.44	0.87
Temperature range 190°-200°C					
A-4	120	0.31	0.47	0.39	0.82
A-13	240	0.33	0.49	0.21	
A-5	479	0.22	0.31	0.22	0.72
Temperature range 235°-245°C					
A-8	161	0.28	0.34	0.22	
A-6	193	0.30	0.37	0.13	1.18
A-9	367	0.21	0.26	0.12	1.98
A-7	1269	0.29	0.04	0.37	38.9
A-10	1850	--	--	--	14.3

Table 5-5. RATIOS OF INDIVIDUAL AMINO ACIDS TO VALINE IN ALIQUOTS OF THE MIOCENE MALAGA MUDSTONE HEATED AT VARIOUS TEMPERATURES.

Sample Number	Time (Min.)	Aspartic Acid	Threonine	Serine	Glutamic Acid	Proline
B-1	(unheated)	0.07	0.79	0.33	0.28	0.58
Temperature range 149°-154°C						
B-2	132	0.04	0.37	0.22	0.16	0.66
B-3	389	0.05	0.33	0.33	0.14	0.81
Temperature range 153°-158°C						
B-4	60	0.07	0.43	0.21	0.10	0.64
B-5	377	0.03	0.28	0.11	0.06	0.36
Temperature range 192°-197°C						
B-6	161	--	0.18	0.14	0.13	0.51
B-18	240	--	0.08	0.08	0.06	0.40
B-7	393	--	--	0.06	0.10	0.41
Temperature range 235°-245°C						
B-11	170	0.04	0.03	0.04	0.02	0.43
B-8	180	--	--	--	--	--
B-13	180	--	--	--	0.27	0.44
B-14	343	0.02	--	0.11	1.15	0.69
B-12	360	0.05	--	--	0.10	0.41
B-9	427	--	0.04	0.07	0.07	0.46
B-10	496	--	0.05	0.10	0.94	0.46
B-15	513	0.04	--	0.29	0.65	0.68
B-16	2597	--	--	--	1.36	1.20

Table 5-5 (continued). RATIOS OF INDIVIDUAL AMINO ACIDS TO VALINE IN ALIQUOTS OF THE MIOCENE MALAGA MUDSTONE HEATED AT VARIOUS TEMPERATURES.

Sample Number	Time (Min.)	Glycine	Alanine	Iso-leucine	Leucine
B-1	(unheated)	1.21	1.83	0.82	1.32
Temperature range 149°-154°C					
B-2	132	1.39	2.10	0.75	1.14
B-3	389	1.96	1.90	1.06	1.25
Temperature range 153°-158°C					
B-4	60	1.02	1.47	0.77	1.19
B-5	377	1.11	1.62	0.63	0.90
Temperature range 192°-197°C					
B-6	161	1.61	2.38	0.77	1.14
B-18	240	1.18	2.08	0.75	1.10
B-7	393	1.42	2.77	0.67	1.01
Temperature range 235°-245°C					
B-11	170	0.78	2.76	0.64	1.04
B-8	180	1.12	2.81	0.54	0.91
B-13	180	1.69	3.29	0.57	0.91
B-14	343	3.36	4.30	0.57	0.79
B-12	360	1.10	2.88	0.25	0.43
B-9	427	3.21	--	0.42	0.79
B-10	496	3.15	4.49	0.48	0.74
B-15	513	3.12	3.07	0.26	0.47
B-16	2597	2.55	2.69	0.26	0.13

Table 5-5 (continued). RATIOS OF INDIVIDUAL AMINO ACIDS TO VALINE IN ALIQUOTS OF THE MIOCENE MALAGA MUDSTONE HEATED AT VARIOUS TEMPERATURES.

Sample Number	Time (Min.)	Tyrosine	Phenyl-alanine	Beta-alanine	Glutamic Acid + Gamma-Amino butyric Acid
B-1	(unheated)	--	--	5.41	0.28
Temperature range 149°-154°C					
B-2	132	0.28	--	3.47	
B-3	389	--	--	2.90	
Temperature range 153°-158°C					
B-4	60	0.12	0.46	0.40	
B-5	377	0.19	0.44	3.38	
Temperature range 192°-197°C					
B-6	161	0.20	0.68	1.26	1.11
B-18	240	0.18	--	1.35	
B-7	393	0.48	0.73	0.29	1.02
Temperature range 235°-245°C					
B-11	170	0.15	0.35	0.18	
B-8	180	1.22	0.27	0.35	1.02
B-13	180	0.24	0.39	0.36	1.38
B-14	343	0.19	0.35	0.41	2.62
B-12	360	0.06	0.08	--	2.77
B-9	427	0.20	0.32	0.31	2.69
B-10	496	0.20	0.34	0.24	7.28
B-15	513	0.10	0.13	0.38	4.01
B-16	2597	0.06	0.05	0.49	

THE USE OF LEUCINE-ISOLEUCINE RATIOS AS AN INDICATION OF THE ORIGINAL AMINO ACID COMPOSITION OF GEOLOGIC MATERIALS

Since leucine and isoleucine are very much alike chemically, they might be expected to react similarly to changes in physico-chemical conditions. Leucine and isoleucine seem to decompose in a parallel manner. See Figure 14. The 0.37 lives for these amino acids are rather similar in pure aqueous solution (Vallentyne, 1964). The proportions of leucine to isoleucine were calculated from the data of this experiment in order to see how closely the two amino acids resemble each other in their response to thermal degradation in the sediments.

Table 5-6, page 89, shows the ratios of leucine to isoleucine for sediments heated at several temperatures. The mean value of the ratios from the Recent sediment is 1.52 with a standard deviation of 0.24; from the Miocene aliquots the mean is 1.56 and the standard deviation is 0.16. The values for samples A-7, A-10, and B-16 were rejected because they were extremely low. The mean values are not very different from the ratios found in the unheated sediments.

The heated samples probably underwent much more severe conditions during the experiment than the Miocene rock was subjected to throughout its entire history. Therefore, the ratio of leucine to isoleucine in the unheated Miocene rock may reflect the value in the original sediment. The ratios in the two unheated sediments are almost identical. The value for the Recent material is 1.63 and that for the Miocene rock is 1.61. This may indicate that the sources of amino acids were similar for the two sediments.

Vallentyne (1964) summarizes amino acid analyses for several groups of organisms. The leucine to isoleucine ratio calculated for algae is 1.67. This is not very different from the values obtained from the sediments. Algal protein may be a major source of the amino acids in the two deposits. The leucine to isoleucine ratios from some possible major sources of sedimentary organic compounds have been calculated from published analyses. Fertility plot soils have ratios which range from 1.21 to 1.37 (Stevenson, 1956). The ratio from the analysis of bacteria and fungi combined is 2.95. The proportion of leucine to isoleucine appears fairly distinctive for many sedimentary organic substances. No data are available for the various classes of terrestrial plants which undoubtedly contribute great quantities of organic debris to basin sediments.

Mixing of various proportions of the several organic products found in marine deposits can give rise to a large range of ratios in sediments. Therefore, it may be significant that the values found in the two sediments studied in this experiment are quite similar. The relative proportions of organic material supplied to the Malaga mudstone in late Miocene time may have been similar to those contributed during the past few thousand years to sediments of the San Pedro Basin. Of course, such a conclusion must be considered as very tentative. Nothing is known at this time about the variation, both vertically and horizontally, of this ratio within the sedimentary units studied in the present experiments. There is also no data on rocks of similar lithologies and different geological ages.

Table 5-6. RATIOS OF LEUCINE TO ISOLEUCINE IN UNHEATED AND HEATED ALIQUOTS OF THE RECENT MARINE SEDIMENT AND THE MIOCENE MALAGA MUDSTONE.

The heating data are to be found in Appendix III.

Recent Marine Sediment		Miocene Malaga Mudstone	
Sample Number	Ratio	Sample Number	Ratio
A-1	1.63	B-1	1.61
A-2	1.43	B-2	1.52
A-3	1.43	B-3	1.18
A-4	1.23	B-4	1.54
A-13	1.55	B-5	1.43
A-5	1.72	B-6	1.48
A-6	1.84	B-7	1.50
A-7	0.04	B-8	1.68
A-8	1.80	B-9	1.88
A-9	1.11	B-10	1.54
A-10	--	B-11	1.63
		B-12	1.72
		B-13	1.59
		B-14	1.38
		B-15	1.81
		B-16	0.50
		B-18	1.47
Arithmetic Mean	1.52	Arithmetic Mean	1.56
Standard Deviation	0.24	Standard Deviation	0.16

KINETICS OF THE THERMAL DECOMPOSITION OF AMINO ACIDS IN SEDIMENTS

The thermal decomposition of various amino acids in aqueous solution has been studied by several investigators (Abelson, 1954; Vallentyne, 1957 and 1964). Abelson studied alanine. Vallentyne studied phenylalanine, pyroglutamic acid, threonine, and serine in detail, and a number of other amino acids in reconnaissance.

Studies by Jones and Vallentyne (1960) of pyrolyzed Pleistocene shell material indicated that the rates of decomposition of some amino acids when heated in shells differed from their decomposition rates measured in aqueous solution. The authors suggested five differences in the chemical environment which may cause the rates of decomposition of amino acids to differ between geologic environments and pure aqueous solutions. They are:

1.) pH conditions

2.) concentration of water

3.) the presence of other compounds with which amino acids can react

4.) the state in which the amino acids exist in the experimental material

5.) the presence of solid surfaces.

All of these differences should apply to the rates of decomposition of amino acids in sediments.

The measurement of amino acids in sedimentary rocks of various geologic ages revealed the presence of some amino acids considered to be relatively unstable to thermal treatment in aqueous solutions. Since

the greatest proportion of the amino acids in sediments and sedimentary rocks is not in the free state it seems reasonable that their decomposition rates might differ considerably from those determined in aqueous solutions.

Furthermore, since some sediments have been subjected to diagenesis for longer periods of time than others the sediments themselves might be expected to differ in some respects. The factors most important to the preservation of the amino acids are probably the degree of stabilization of the organic material in the rock and the relationship between the amino acids and the clays of the rock.

Two fine-grained clastic sediments deposited in more or less similar basins of deposition would be expected to contain a large number of constituents in common. Any disparity in the decomposition of amino acids between the two sediments would have to be due to some major difference in the rocks themselves; i.e., geologic age, diagenetic history, and the presence of large concentrations of some constituent not common to them both. One may neglect the many dissimilarities existing between a simple aqueous solution and a sedimentary rock by comparing a Recent sediment and an ancient sediment.

It is believed, by some, that amino acids are not linked by peptide bonds in older sediments. An interesting study by Abelson (1955) has shown that peptide bonds are almost quantitatively broken in Pleistocene fossils where water is available. He further demonstrated that no peptide bonds remain in Miocene fossils which he studied. Since amino acids seem to persist in fine-grained clastic sediments for long periods of time, it follows that they must be bound

in some manner to the organic matrix or the minerals of the sediments. It is not known what effect this bonding may have upon thermal stability, but it is believed to have a marked effect on the geologic stability of the amino acids.

In most studies performed to date it has been assumed that thermal decomposition is the primary means by which the measurable concentrations of amino acids in sedimentary rocks are reduced. It is quite possible that thermal decomposition is the main process by which organic constituents are eliminated from sedimentary rocks. However, the fact that other organic constituents, previously unsuspected, have been won from geologic materials by drastic chemical treatment indicates that geochemical processes may effectively hide some organic constituents from our observation.

The majority of the amino acids in the Recent sediment is believed to be present as peptides, proteins, or free amino acids. The remainder is probably bound in the humic acid fraction and in other organic complexes as well as adsorbed to clay minerals. Most of the amino acids in the Recent sediment are expected to be easily accessible to acid hydrolysis. The amino compounds in the Miocene rock are probably not in proteinaceous material.

Two sediments, one Recent and the other Late Miocene, were heated in the presence of water to determine whether the thermal decomposition rates of the amino acids available to 6 N HCl are affected by differences in the geochemical environment caused by diagenesis. The thermal decomposition reactions of several of the amino acids in pure solution have been found to be first order. Because of many

difficulties encountered in the heating experiments, most important of which is the thermostatic control, it was not possible to determine the reaction kinetics of amino acids in sediments with a reasonable degree of confidence. Sometimes several runs had to be made before getting good agreement between the maximum temperatures.

It is evident from the experiments that thermal decomposition is not the only process affecting the measurable concentrations of amino acids in the heated samples of the Miocene rock. Degradation of the amino compounds in the Recent sediment appears more regular. Thermal decomposition reactions are generally assumed to be first order. When the logarithms of the measured concentrations of the amino acids are plotted as a function of heating time in the temperature range of 235° to 245°C, the points fall rather close to a straight line. This indicates that the thermal degradation of the amino compounds in the Recent sediment may be first order with respect to these substances. Amino acids are present in the sediments in very small amounts. Therefore, the concentrations of the amino compounds probably control their rate of decrease. This may be true despite the fact that factors other than simple thermal decomposition may alter amino acids in this sediment.

Amino acids are known to react with reducing sugars present in solution. This is the well known Maillard reaction, which produces the browning of food. Basic amino acids are most affected by this reaction. In the sediments any reaction with reducing sugars is probably not first order since the sugars are generally found in concentrations comparable to those of the amino compounds (Degens et al., 1961, 1963).

Reaction with atmospheric oxygen is known to reduce the yields of amino acids during heating. Therefore, every effort was made to exclude oxygen from the bombs during the experiments. The reaction would probably not be first order if oxygen and the amino compounds were present in comparable concentrations. However, other organic constituents, more abundant than the amino acids, may be expected to react with a large proportion of any oxygen present.

Catalysis by the active surfaces of clay minerals and the metal of the bomb may affect the decomposition rates of the amino compounds in solution. If the available surface area were very large and the amino acid concentrations low, then the reaction should still be first order with respect to the amino compounds.

With the exception of atmospheric oxidation, the other influences described above may be expected to affect the amino acids in the diagenetic environment during heating. Thermal degradation in pure aqueous solutions provides data for an upper limit for the time of preservation of free amino acids under clean conditions. The rates of disappearance of the amino acids measured in the sediments during this experiment indicate how rapidly these substances may be destroyed under severe conditions in the natural environment. This probably applies to those mobile amino compounds in the Recent sediment.

In order to estimate the activation energy for the decomposition of alanine in the Recent sediment, it is assumed that the thermal degradation reaction is first order. The reaction rate constants were computed for samples heated at three well-separated temperatures. The logarithms of the computed rate constants were plotted as a function of

the reciprocal of the Kelvin temperature. These temperatures are the midpoints of the temperature ranges of the sample runs. See Figure 15, page 96. The data scatter considerably. Nevertheless, a straight line can be drawn through points for each temperature. This is the line of greatest slope which may be obtained from the data. It may be a limiting line. An apparent activation energy of 8400 calories per mole is calculated from the slope. This is considerably lower than the value of 44,000 calories per mole determined by Abelson (1954) for the thermal decomposition of alanine in pure aqueous solutions.

The low value of the apparent activation energy indicates that surface catalysis may affect the rate of decomposition of alanine in the sediments. In general, the activation energies for the oxidation of organic compounds range from 15,000 to 25,000 calories per mole. The low value for the apparent activation energy indicates that atmospheric oxidation is probably not important in this experiment.

Vallentyne (1964) in his description of the thermal decomposition of serine indicates that this amino acid breaks down by three pathways. Decarboxylation is the major pathway at the temperatures studied. Deamination takes place to some extent. Also serine converts to glycine and alanine. However, the overall reaction is apparently first order. In the case of another amino acid, alanine, it was found that the only pathway of thermal decomposition was by decarboxylation. This undoubtedly accounts, in part, for its much greater stability than serine.

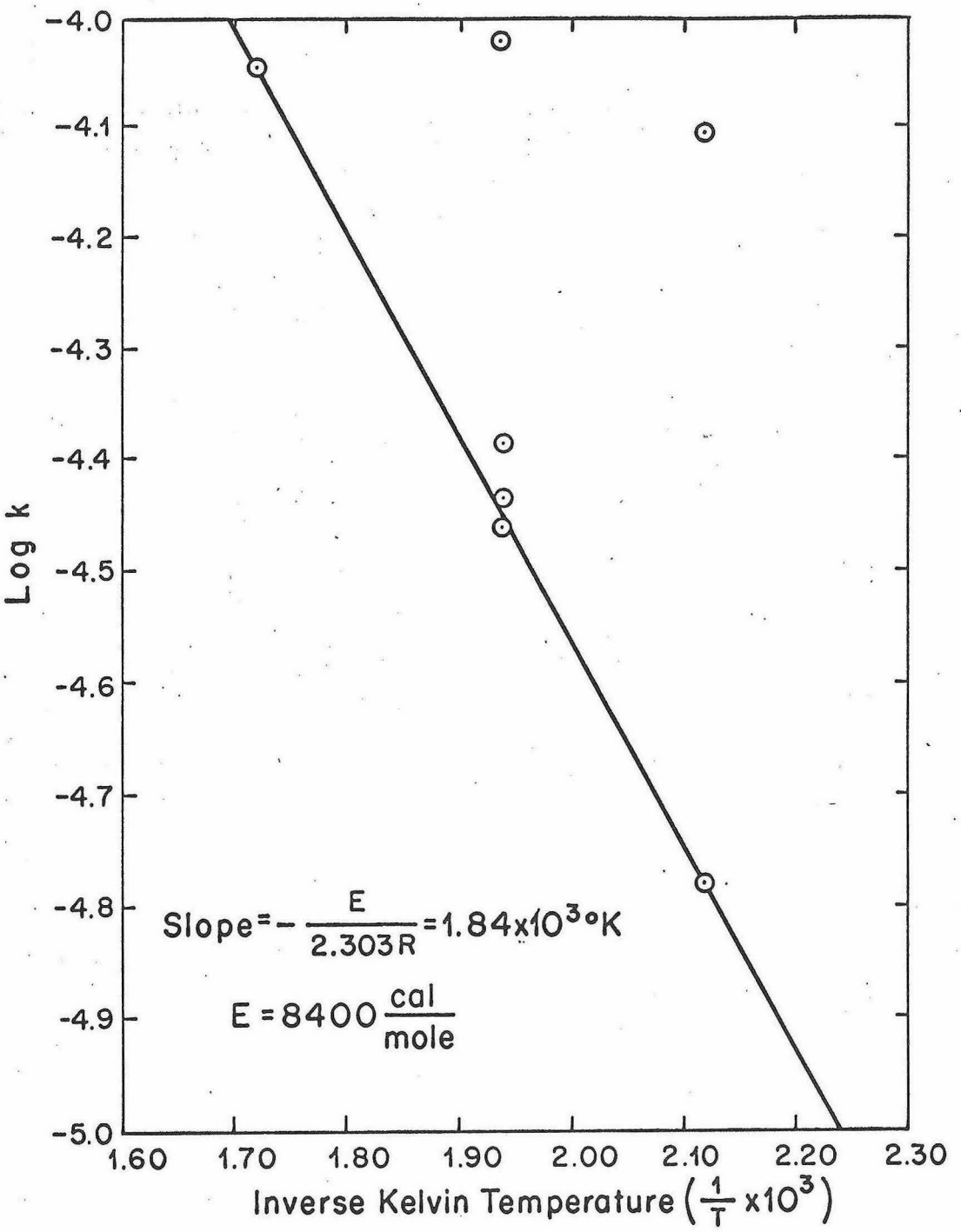

Figure 15. The variation of the logarithm of the reaction rate constant for the decomposition of alanine in a Recent marine sediment as a function of the inverse Kelvin temperature. The apparent activation energy for the reaction is computed from the line of maximum slope.

EFFECT OF PROLONGED HYDROLYSIS ON A HEATED MIOCENE MUDSTONE SAMPLE

Three aliquots of the Malaga mudstone heated under similar temperature conditions, but subjected to different times of heating and hydrolysis, are compared in Table 5-7 on page 98. It can be seen that the various amino acids behave differently and in more or less consistent fashion when the time of heating or hydrolysis is changed. All of the amino compounds except the aliphatic amino acids, glucosamine, and beta-alanine show a substantial decline after heating for longer periods at similar temperatures. This can be seen by comparing samples B-4 and B-5. The apparent increase of the aliphatic amino acids may be due to their greater stability in hot aqueous solution and to their greater relative abundance in the organic fraction of sedimentary rocks.

Continued refluxing appears to release more amino acids from the organic complex present in the rock. This may be seen by comparing aliquots B-4 and B-17 which were heated at the same temperature for about the same time. The only major difference in treatment is that B-17 was hydrolyzed for three days instead of one. All of the amino compounds listed show significant increases except aspartic acid, serine, and tyrosine. Aspartic acid does not appear to change. Serine and tyrosine show significant decreases with continued hydrolysis. It is important to note that both of these amino acids have exposed hydroxyl groups on their sidechains. It is a well known fact that serine is altered during acid hydrolysis; however, no data are available on tyrosine. The concentration of tyrosine seems to change significantly with continued hydrolysis. If tyrosine is altered by loss of the

Table 5-7. CONCENTRATIONS OF AMINO COMPOUNDS IN COMPARABLE PORTIONS OF THE MALAGA MUDSTONE SUBJECTED TO SLIGHTLY DIFFERENT TREATMENTS; CONCENTRATIONS IN MICROMOLES PER GRAM SEDIMENT.

Amino Compounds	B-4 154° C 60 minutes Hydrolyzed 1 day	B-5 153°-158° C 377 minutes Hydrolyzed 1 day	B-17 154° C 70 minutes Hydrolyzed 3 days
aspartic acid	0.0073	0.0037	0.0072
threonine	0.0439	0.0372	0.0627
serine	0.0212	0.0150	0.0110
glutamic acid	0.0105	0.0079	0.0283
proline	0.0648	0.0480	0.0820
glycine	0.104	0.150	0.268
alanine	0.150	0.218	0.218
valine	0.102	0.135	0.152
isoleucine	0.0782	0.0848	0.130
leucine	0.121	0.121	0.166
tyrosine	0.0122	0.0262	0.0046
phenylalanine	0.0467	0.0590	0.0905
glucosamine	0.0372	0.372	0.0555
beta-alanine	0.0403	0.456	0.155

hydroxyl group during hydrolysis, part of the increase of phenylalanine may be accounted for in this way. Serine may be altered to alanine or glycine by a similar process. Finely divided sheet structure silicates which are altered by heating and acid-leaching may affect the transformation of the hydroxyl-bearing amino acids by surface catalysis.

Although threonine is also known to be affected by acid hydrolysis, its hydroxyl group is not as well-exposed as that of serine and tyrosine; therefore, one would expect surface catalysis to be less effective if it is the process which is active in the destruction of serine and tyrosine. Such an effect should be studied using known quantities of the three amino acids.

UNKNOWNS FOUND IN THE EXPERIMENTS

Several tentatively identified unknowns were found in the heated and unheated specimens. A peak with an R_f value very similar to hydroxylysine was found in both the unheated and heated specimens. The substance producing this peak seems rather stable.

A peak which may correspond to ethanolamine is found in the chromatograms of the unheated samples; this peak becomes larger after heating. It is particularly abundant in the heated samples of the humic acids. Ethanolamine is known to be one of the decomposition products of serine.

A peak ascribed tentatively to alloisoleucine is found in the heated sediment samples. It appears to grow more abundant with contiued heating. It may result from a change in configuration of isoleucine during heating or during release from the organic matrix. Alloiso-

leucine has been found to be present in polymixins which are antibacterial agents produced by certain bacteria and fungi (Fruton and Simmonds, 1958). Since bacteria and fungi are abundant in soils and sediments this amino acid might be expected in sedimentary organic matter.

THERMAL STUDIES OF THE AMINO ACIDS IN THE HUMIC ACID FRACTIONS OF A RECENT MARINE SEDIMENT AND A PORTION OF THE MIOCENE MALAGA MUDSTONE

The method used to obtain the humic acid fractions of the two rocks is described in Chapter 2. Both extracts were treated similarly in order to get comparable results.

Humic acid extracts from various sources have been analyzed for amino acids by several investigators. The sources include soils of every type, Recent lake and bog sediments, and peat deposits. This work has been summarized by Swain (1963). Degens et al. (1963) studied the humic acid extract of a Recent sediment sample from the San Diego Trough.

Among the amino acids reported by the investigators who have studied soil humic acids are cysteic acid, methionine sulfoxide, methionine sulfone, hydroxyproline, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, one-half cystine, valine, methionine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine, alpha-aminobutyric acid, alpha-epsilon diaminopimelic acid, and dihydroxyphenylalanine (Dopa). Of these all but alpha-aminobutyric acid, alpha-epsilon-diaminopimelic acid, and dihydroxyphenylalanine have been found in acid hydrolyzates of normal proteins. However, cysteic acid, methionine sulfoxide, and methionine sulfone are believed to be oxidation products of the amino acids methionine and cysteine. It is notable that the amino acid, beta-alanine, and the aminosugar, glucosamine, have not been reported in soil humic acids. These amino compounds are very abundant in the humic acids of the two marine rocks

analyzed in this study.

Aspartic acid, threonine, glutamic acid, glycine, alanine, valine, the leucines, phenylalanine, lysine, and histidine have been reported by Blumenthals and Swain (1956) and Swain et al (1959) in the humic acid extracts of lake sediments and peat.

Degens found almost all of the amino acids normally found in acid hydrolyzates of proteins in a Recent sediment humic acid (personal communication). He concluded that the relative proportions of amino acids found in the humic acid extract were not the same as for the total sediment from which the humic acids were extracted. He finds that the amino acid spectrum from the humic acid fraction resembles that of the Miocene sediment found near the bottom of the experimental Mohole core (Rittenberg et al., 1963). In all of the above-mentioned studies the amino acids were measured in the initial HCl extract of the humic acid. Essentially all of the analyses of humic acids for amino compounds carried out prior to the present study were done by paper chromatography or by wet chemical techniques.

It is not known how amino acids are bound in humic acids, nor what role the amino compounds play in the formation of humic acids. An appreciable amount of nitrogen has been found in humic acid extracts of natural samples. Since amino acids are reactive molecules and they have been found in HCl hydrolyzates of humic acids, it is believed by some that amino acids may form an integral part of the humic acid polymer. Trautner and Roberts (1950) believe that amino acids react with quinones to form amino phenols which become polymerized to form nitrogen-bearing humic acid polymers. Flaig (1964) describes experiments in which amino

acids were joined to the 1,2-diphenol, catechol, in the presence of enzymes (phenoloxidases). The natural source of the phenolic reactants is the lignin fraction of terrestrial plants.

Humic acids are precipitated by acidification of the sodium hydroxide solution used to extract them from the sediments. It is possible that amino acids or peptides free in solution are adsorbed to or react with the humic acid precipitate. Amino acids in acidic solution are cationic in nature. The base exchange properties of humic acid materials are well known. If amino acids are only loosely held to humic acids in sediments, then one would expect them to be removed through geologic time under relatively mild conditions. However, if the amino acids function as part of the integrated polymer of humic acids, one would expect them to be stabilized to some degree. Therefore, the removal of amino acids from sedimentary rocks would require more rigorous conditions than those necessary for the removal of amino acids from fossil shells.

The polymeric nature of the humic acids and the fact that they constitute a large proportion of the organic fraction of sediments has led some to suggest that these substances are the precursors of the insoluble, relatively inert organic material called kerogen. Since our knowledge of the chemical constitution of kerogen is very sparse, such a suggestion is at best very tentative. During geologic time one would expect any humic acid materials deposited in the sediments to lose those constituents or functional groups which are least stable chemically as well as those which are loosely held. The carbon-nitrogen ratios of various kerogens differ significantly from the

carbon-nitrogen ratios of sediment and soil humic acids.

THE AMINO ACIDS RELEASED BY 6 N HCl HYDROLYSIS OF UNHEATED HUMIC ACID EXTRACTS

One hundred milligram aliquots of the humic acid extracts of both sediments were refluxed with 6 N HCl for twenty-four hours. The concentrations of the acidic and neutral amino acids and aminosugars are given in Tables 6-1 and 6-2 which can be found on pages 105 and 106. Analyses C-1 and D-1 represent, respectively, the unheated humic acid extracts from the Recent marine sediment and the Miocene Malaga Mudstone.

Other portions of the two humic acids were refluxed twice and the second 6 N HCl extract was analyzed in order to see whether continuous yields of amino acids could be obtained. The concentrations of amino acids measured were negligibly small in comparison to those obtained from blank runs; therefore, they were not tabulated. The residues of humic acid recovered after the initial hydrolysis were not very large (34 milligrams of the Recent sediment humic acid and 96.2 milligrams of the Miocene sediment humic acid). This may account in part for the low yields.

Analyses C-1 and D-1 indicate that all of the acidic and neutral amino acids which generally occur in proteins are present in the humic acid extracts of both sediments. The acid hydrolyzate of the Recent humic acid contained all of the amino acids except methionine in concentrations of one or more micromole per gram of humic acid. The yields of the amino acids from the Miocene humic acid range from 0.13

Table 6-1. CONCENTRATIONS OF ACIDIC AND NEUTRAL AMINO ACIDS IN A HUMIC ACID EXTRACT OF A RECENT MARINE SEDIMENT (micromoles per gram of humic acid).

L = probable low value

	Recent Sediment Humic Acid Sample Number		
Amino Acid	C-1	C-2	C-3
hydroxyproline	--	2.25	--
aspartic acid	9.00	1.31	0.255
threonine	10.50	2.22	0.189
serine	8.36	4.04	0.223
glutamic acid	11.30	15.00	1.74
proline	10.70	10.90	0.200L
glycine	28.10	33.80	4.82
alanine	14.40	16.20	1.84
one-half cystine	1.00	1.27	--
valine	7.86	8.20	1.91
methionine	0.27	0.36	--
isoleucine	5.04	4.34	0.661
leucine	7.22	6.21	0.866
tyrosine	2.54	2.47	0.060L
phenylalanine	5.06	4.20	0.153L
glucosamine	8.68	--	--
beta-alanine	6.27	1.60	0.094L
galactosamine	5.66	--	--

Table 6-2. CONCENTRATIONS OF ACIDIC AND NEUTRAL AMINO ACIDS IN A HUMIC ACID EXTRACT OF THE MIOCENE MALAGA MUDSTONE (micromoles per gram of humic acid).

	Miocene Malaga Mudstone Humic Acid Sample Number			
Amino Acid	D-1	D-2	D-3	D-4
aspartic acid	1.84	0.225	0.0462	0.155
threonine	1.18	0.323	0.0074	0.174
serine	1.33	0.350	0.118	0.106
glutamic acid	1.74	2.32	2.62	0.446
proline	1.37	1.09	0.735	0.340
glycine	3.60	1.84	1.76	2.39
alanine	2.87	1.94	1.55	0.364
one-half cystine	0.13	--	0.150	--
valine	1.74	1.15	0.830	0.530
methionine	0.69	--	0.070	0.086
isoleucine	0.70	0.365	0.258	0.179
leucine	1.39	0.653	0.343	0.232
tyrosine	*	0.023	0.103	0.029
phenylalanine	12.42*	0.041	0.080	0.038
glucosamine	*	--	--	--
beta-alanine	11.43	0.392	0.125	0.071

*Value so marked represents total of those amino acids marked with an asterisk.

to 11.43 micromole per gram of humic acid. The basic amino acids are not listed, but their yields are comparable to those of the amino compounds tabulated for the two sediment humic acids. Among the basic amino acids definitely identified were ornithine, lysine, and arginine. A peak which has an R_f value very similar to that of hydroxylysine is also found in chromatograms of both sediment humic acids.

Beta-aminoisobutyric acid, gamma-aminobutyric acid, and histidine are probably present; however, their corresponding peaks on the chromatogram seem to have been interfered with by other peaks which run near them. They appear as shoulders or as part of obviously broadened peaks. Their presence will have to be verified by other analyses.

Beta-alanine and methionine are yielded in greater absolute amounts from the Miocene sample than from the Recent sample. It is also interesting to note that glycine and alanine are the most abundant of the normal protein amino acids found in the two humic acid extracts. Aspartic acid, threonine, serine, glutamic acid, proline, valine, and leucine are next in order of abundance although not in the order named. Glucosamine is the most abundant amino compound not found in proteins that is found in the Recent humic acid. Since glucosamine, tyrosine, and phenylalanine did not separate during the analysis of the unheated Miocene humic acid, it is not possible to say how much glucosamine is present in the sample. However, the peak on the chromatogram appears to be predominantly glucosamine, and this peak has swamped both the tyrosine and phenylalanine peaks. Therefore, it is possible that glucosamine is also very abundant in the unheated humic acid extract of the Miocene Malaga mudstone. Other tests will have to be performed in

order to verify this. The fact that glucosamine is rather unstable to heating permits the peaks of tyrosine and phenylalanine to be separated in chromatograms from heated samples of the Malaga mudstone humic acid.

Galactosamine, another amino sugar, occurs in the Recent humic acid but does not appear to be present in the Miocene humic acid.

THE AMINO ACIDS FOUND IN 6 N HCl HYDROLYZATES OF HEATED ALIQUOTS OF THE SEDIMENTARY HUMIC ACIDS

One hundred milligram aliquots of the two humic acid extracts were heated with water in a bomb for periods of four hours at two widely differing temperatures. The Recent sediment humic acid extract samples reached maximum temperatures of 201°C and 475°C. The Miocene sediment humic acid extract samples reached maximum temperatures of 193°C and 514°C. After refluxing with 6 N HCl the samples were analyzed for amino acids. The higher temperature samples yielded concentrations of amino acids lower than blank runs. Therefore, the values are not tabulated. Sample C-2, Table 6-1, shows the amino acid concentrations found in the Recent sediment humic acid heated to 201°C. Sample D-2, Table 6-2, gives the amino acid concentrations for the Miocene humic acid heated to 193°C. Sample D-3 shows the hydrolyzable amino acids from an aliquot of the Miocene humic acid heated for 508 minutes at 241° to 245°C. Total heating time was 686 minutes.

Comparison of the heated specimens, C-2, D-2, and D-3, with their unheated counterparts, C-1 and D-1, reveals that some of the amino acids are found in increased yields after continued heating.

In the Recent sediment humic acid extract all of the amino

acids except glutamic acid, proline, glycine, alanine, one-half cystine, methionine, and valine decrease with heating. Of those that decrease only aspartic acid, threonine, serine, and beta-alanine appear to decrease markedly during the four hour heating period. The others decrease only slightly during this time. The amino acid, hydroxyproline, appears in the heated specimen of the Recent sediment humic acid. Since hydroxyproline has very nearly the same R_f value as aspartic acid, it is very likely that the large aspartic acid peak which appeared on the chromatogram of the unheated Recent humic acid, C-1, masked the more feeble hydroxyproline peak. Glutamic acid, glycine, alanine, and valine are found in significantly greater yields after heating. Proline shows very little change with heating.

All of the amino compounds except glutamic acid, tyrosine, and phenylalanine show a decrease in the Miocene humic acid with continued heating. However, serine, proline, glycine, alanine, valine, isoleucine, leucine, and beta-alanine show only a slight decline after continued heating. Aspartic acid and threonine seem to decrease rather rapidly by comparison with the other amino acids. Glutamic acid displays a striking increase with time of heating which probably cannot be attributed solely to its well known thermal stability (Vallentyne, 1964; Povoledo and Vallentyne, 1964). Apparently glutamic acid is a major constituent of the amino acid fraction of the Miocene humic acid extract.

AMINO ACIDS STRUCTURALLY HELD IN HUMIC ACIDS

There is a question as to how amino acids occur in the humic acids. Amino compounds may become a part of the humic acid polymer by occlusion or surface adsorption during precipitation. Amino acids may also form an integral part of humic acids. The experiment described below was performed in order to get information useful to the resolution of this question.

Both humic acid extracts were refluxed for twenty-four hours with 6 N HCl. This process should remove any amino compounds adsorbed to surfaces of the humic acid extracts, and a large proportion of those which may be bound to side groups of the polymer. One hundred milligram aliquots of each of these previously hydrolyzed humic acid extracts were subjected to heating in a bomb with water for four hours. This step might be expected to disrupt the humic acid polymer and expose internally held amino acids to HCl hydrolysis. The maximum temperature attained by the Recent humic acid was 197°C and that for the Miocene humic acid was 192°C. The heated extracts were refluxed with 6 N HCl and the hydrolyzates were analyzed. Analysis C-3 in Table 6-1 gives the amino acid yields for the Recent humic acid. Analysis D-4 in Table 6-2 shows the corresponding concentrations for the Miocene humic acid.

As discussed previously, a second refluxing with 6 N HCl yielded insignificant amounts of amino acids from the unheated humic acids. Therefore, virtually all of the amino acids available to HCl hydrolysis had been removed from the humic acid by the initial HCl extraction. Heating in the presence of water for four hours at tempera-

tures around 200° C followed by refluxing with 6 N HCl for another twenty-four hours released significant quantities of amino acids similar to those observed in previous hydrolyzates. Glutamic acid, glycine, alanine, valine, isoleucine, and leucine are the most abundant amino compounds in both of the humic acid extracts. However, aspartic acid, threonine, and serine, which are known to be rather unstable to heating, are released in rather significant amounts from both sediment humic acids. Threonine occurs in approximately the same abundance in both of the humic acids. Serine is found in much smaller concentrations. How the amino compounds are bound in the humic acid complex is not known. The rates at which amino acids decomposed during the heating process are not known. It is reasonable to suppose, however, that the relative proportions of the amino acids in the analyses, C-3 and D-4, reflect the concentrations of these substances in the humic acids unless those amino compounds with reactive side groups are more firmly held in the polymer. The data from this experiment suggest that amino acids form an integral part of the humic acids of sediments. The actual concentrations of individual amino acids in the humic acid extracts are still not known.

The amino acids in analyses C-3 and D-4 are arranged in order of decreasing abundance in Table 6-3 on the next page. The peaks for proline, phenylalanine, and beta-alanine on the chromatograms for sample C-3 did not appear to reach their full height. Therefore, the measured values are probably low.

If the two lists are compared, it can be seen that the relative measured concentrations are similar. This may indicate that the amino acids in the humic acid fraction of the Miocene marine

sediment have been little altered through time. It is also possible that the humic acids of both sediments have similar origins.

Table 6-3. AMINO ACIDS FOUND IN HUMIC ACID EXTRACTS AFTER 6 N HCl HYDROLYSIS AND HEATING.
Amino compounds are arranged in decreasing order of abundance.

Recent Humic acid (Sample C-3)	Miocene Humic Acid (Sample D-4)
glycine	glycine
valine	valine
alanine	glutamic acid
glutamic acid	alanine
leucine	proline
isoleucine	leucine
aspartic acid	isoleucine
serine	threonine
proline	aspartic acid
threonine	serine
phenylalanine	beta-alanine
beta-alanine	phenylalanine
tyrosine	tyrosine

The leucine-isoleucine ratios for samples C-3 and D-4 are essentially identical. The value for C-3 is 1.31 and for D-4 is 1.32. These values are also very similar to those for the amino acid fraction of fertility plot soil organic matter which range from 1.21 to 1.37. Perhaps the humic acids found in the basin sediments were formed in soils.

THE PROBLEM OF CONTAMINATION

In order to show that the amino compounds found in the Miocene Malaga mudstone and its humic acid extract have actually persisted since Miocene time, it is necessary to demonstrate that the amino acids found do not represent natural contamination. Laboratory contamination was negligible as determined by blank runs. All of the data were reduced by the amount of amino acids determined in the blanks.

It is not so easy to demonstrate that the Miocene rocks have not been contaminated by amino acids since Miocene time. However, the location from which the sample was taken was chosen in order to minimize the danger of Recent contamination. See Chapter 2.

DeVries (1959) in his discussion of carbon-14 age dating enumerates a number of cases in which it was found that materials used for age dating had been impregnated with humic substances which had seeped downward from younger sediments. The carbon-nitrogen ratios of soil humic acids range from 8 to 12 depending upon the maturity of the humic acid. Not all of this nitrogen is present in the form of amino acids. Amino acids probably do not constitute a large fraction of the organic material in contaminating solutions.

Since a large proportion of the organic fraction of the Miocene sample was extracted as humic acid, it was felt necessary to check for carbon-14 activity in the humic acid extract. A large amount of the humic acid extract was sent to Dr. David Thurber at the Lamont Geological Observatory for dating. Through an accident at the laboratory, the sample was lost and insufficient material was available for

a second determination. One hundred grams of the total rock sample were sent for carbon-14 analysis. The carbon from fifty grams of the rock yielded no measurable carbon-14 activity. The sample represented more than three grams of carbon. See analyses in Table 6-4 on the following page. Since the carbon in the humic acid extract represents 7.6% of the total organic carbon of the rock and it is likely that any Recent carbon contamination would be in the humic acid fraction, it appears that no appreciable Recent contamination has occurred. This does not exclude the possibility that the rock was contaminated some time between the Miocene and 22,000 years ago. This assumes, of course, that all of the extractable humic acid represents contamination. On the basis of available data it appears that the Miocene sediment is uncontaminated by Recent carbon.

THE EFFECT OF HEATING ON THE CARBON, HYDROGEN, AND NITROGEN CONTENT OF HUMIC ACIDS AND SEDIMENTS

Table 6-4, on the following page, shows the organic carbon, hydrogen, and nitrogen contents of the unheated sediments and humic acids used in this experiment. It also contains the carbon-nitrogen ratios of the various materials. The humic acids are relatively higher in nitrogen and lower in hydrogen than the organic fractions of the total sediments. The higher carbon-nitrogen ratios of the Miocene sediments and humic acids may indicate the greater diagenetic maturity of these materials.

Tables 6-5 and 6-6, pages 117 and 118, show the carbon-nitrogen and carbon-hydrogen mole ratios of the sediments and humic acids subjected to various heating and hydrolysis treatments. The results for the heated and hydrolyzed samples are somewhat erratic. Nitrogen and hydrogen are lost relative to carbon with repeated hydrolysis and more severe heating. The ratios change more regularly in the Miocene materials than in the Recent sediment. This may also reflect the diagenetic maturity of the Miocene sediment.

In general, the carbon-nitrogen and carbon-hydrogen ratios of the two humic acids vary in a parallel manner with heating and hydrolysis although the initial values are different. The low value of the carbon-nitrogen ratio in sample C-4 is unexplained.

The ratios in the overall organic fractions vary similarly in the two sediments. The carbon-hydrogen ratio decreases sharply in both sediments heated around 500° C. This may indicate that extensive decarboxylation has taken place. The decrease in measurable amino compounds

Table 6-4. ORGANIC CARBON, HYDROGEN, AND NITROGEN CONTENT OF THE UNHEATED SEDIMENTS AND THE HUMIC ACIDS EXTRACTED FROM SEDIMENTS USED IN THE HEATING EXPERIMENTS (Wt. %).

	C	H	N	Weight $\frac{C}{N}$	Atomic $\frac{C}{N}$	% Sediment Carbon in Humic Acid	% Sediment Nitrogen in Humic Acid
Recent Marine Sediment	3.66	0.87	0.29	12.6	14.7		
Recent Sediment Humic Acid	16.31	3.21	1.65	10.23	11.93	36.9	54.3
Miocene Malaga Mudstone	6.29	0.82	0.42	15.2	17.7		
Malaga Mudstone Humic Acid	35.92	4.19	2.50	14.36	16.74	7.6	8.1

and the increase of the carbon-nitrogen ratio indicate that nitrogen is lost in some form other than amino acids. Considerable amounts of gas were evolved in the heating experiments. The compositions of the gases produced were not determined since facilities for doing gas analyses were not available. The odors of H_2S or mercaptans and of phenol were detectable when the bombs were opened. The odor of phenol was also noticeable in most of the HCl extracts. The general increase of the carbon-hydrogen ratio may result from the release of methane.

The carbon-nitrogen ratio is greater than 26 in the strongly heated Miocene sediment and humic acid residues. This value is greater than the carbon-nitrogen ratios of some kerogens (Forsman, 1963). The processes of short-term heating and acid leaching may simulate, but not necessarily duplicate, the long-term geologic processes which eventually produce kerogen.

Table 6-5. CARBON-NITROGEN AND CARBON-HYDROGEN MOLE RATIOS OF THE RECENT AND MIOCENE HUMIC ACIDS SUBJECTED TO HEATING AND 6 N HCl HYDROLYSIS.

Recent Marine Sediment Humic Acid	Max. Temp. (°C)	Total Time (hours)	Hydrolyzed	$\frac{C}{N}$	$\frac{C}{H}$
Untreated Humic Acid	unheated	0	No	11.93	0.428
C-1 residue	unheated	0	Yes	14.36	0.568
C-5 residue	unheated	0	Twice	15.57	0.636
C-2 residue	201	4	Yes	14.38	0.641
C-4 residue	475	4	Yes	11.30	0.671
Miocene Malaga Mudstone Humic Acid					
Untreated Humic Acid	unheated	0	No	16.74	0.719
D-1 residue	unheated	0	Yes	21.54	0.865
D-6 residue	unheated	0	Twice	22.01	0.848
D-2 residue	193	4	Yes	19.66	0.885
D-5 residue	510	4	Yes	26.30	1.232

Table 6-6. CARBON-NITROGEN AND CARBON-HYDROGEN MOLE RATIOS OF THE RECENT SEDIMENT AND MIOCENE MALAGA MUDSTONE SUBJECTED TO HEATING AND 6 N HCl HYDROLYSIS.

Recent Marine Sediment	Max. Temp. (° C)	Total Time (hours)	Hydrolyzed	$\frac{C}{N}$	$\frac{C}{H}$
Untreated Sediment	unheated	0	No	14.7	0.353
A-1 residue	unheated	0	Yes	19.6	0.380
A-13 residue	198	4	Yes	16.7	0.383
A-14 residue	473	4	Yes	20.3	0.179
Miocene Malaga Mudstone					
Untreated Sediment	unheated	0	No	17.7	0.644
B-1 residue	unheated	0	Yes	22.2	0.755
B-18 residue	194	4	Yes	23.8	0.732
B-22 residue	470	4	Yes	26.1	0.451

CONCLUSIONS

The Miocene rock and humic acid extract, despite their great age, yield essentially the same amino compounds found in the Recent materials. The quantities of amino acids found in the HCl extract of the Miocene sediment are one to two orders of magnitude lower than those in the Recent sediment hydrolyzate. Such thermally unstable amino compounds as aspartic acid, threonine, serine, cystine, methionine, arginine, and glucosamine have apparently survived since Late Miocene time in the Malaga mudstone. On the basis of the available evidence no Recent contamination is indicated.

Humic acids were obtained from the Recent sediment in greater quantities than from the Miocene rock although the latter contains a higher concentration of organic matter. Diagenesis has apparently affected the mobility of the humic substances in the Miocene deposits. The yields of amino acids from the humic acid extract of the Miocene sediment are diminished slightly compared to those of the Recent sediment humic acid. This may reflect a difference in the humic acid extracts caused by age.

The humic acids from the two sediments apparently contain amino compounds in their interiors. The order of abundance of the internally held amino acids is similar in the two humic acid extracts. Therefore, the humic acids seem to be very much alike despite their age differences. The fact that the internal constitutions of the humic acids are similar may indicate that the amino acids are, indeed, a structural part of the humic complex. Aspartic acid, threonine, and

serine are obtained in significant yields from the humic acid extracts after previous HCl hydrolysis and heating. Therefore, it should not be surprising to find these amino acids in the hydrolyzates of older sedimentary rocks. The leucine-isoleucine ratios are identical for the internally held amino acids of the humic acid complexes of both sediments. The similarity of these ratios to those from fertility plot soil organic matter may indicate a continental source for the humic acids.

Very little is known about the amino acid composition of complete organisms or groups of organisms which may contribute biochemical compounds to sediments. It is not possible to determine with certainty the origin of sedimentary amino acids. The leucine-isoleucine ratios of the two sediments are almost identical. Although they are slightly lower, the ratios are very similar to the value measured for algae. The values of this ratio in terrestrial green plants are not known. Nearshore basin sediments may receive large amounts of organic matter from terrigenous sources. The land areas draining into the Southern California basins have a semi-arid climate. Upwelling is common in the offshore waters. Therefore, a large proportion of the organic matter in the basin deposits is probably derived from marine plants.

The heating experiments indicate that there is a fundamental difference in the way amino acids are combined in the organic fractions of the two sediments. The data are consistent with the concept that the Recent sediment has at least two major repositories for amino compounds which may be chemically quite different. These

reservoirs might be expected to consist of proteinaceous material containing peptide bound amino acids and humic acids or other hetero-polycondensates, which are not appreciably soluble in near-neutral aqueous solutions. Amino acids in the heated Miocene rock behave as if they are part of the more stable type of repository. Although the data are suggestive they cannot be considered conclusive, since much remains to be learned about the nature of the various organic fractions of sediments.

In general, all of the amino acids decrease with heating in the Recent sediment. Around 240° C a large proportion of the amino compounds in this sediment is rapidly altered. Measurable yields of many of the amino acids are obtained after longer periods of heating at this temperature. Increased yields of glutamic acid are found after prolonged heating. Gamma-aminobutyric acid also increases to some extent after longer heating intervals.

Under similar heating conditions the amino acids in the Miocene rock generally decrease less rapidly than their counterparts in the Recent sediment. Proline and valine diminish very gradually with heating time. Glutamic acid, glycine, and alanine are obtained in greater yields from some of the heated samples than from the unheated rock. Heating around 150° C for longer periods results in larger yields of most of the amino compounds from the Miocene sediment. This probably does not apply for very long heating periods.

It would appear, therefore, that the amino acids in the Recent sediment are less stable thermally than the amino compounds in the Miocene rock. Heating the sediments in a bomb with water alters

the more stable organic complexes and may permit greater quantities of amino acids to be extracted during HCl hydrolysis. The increased yields of glutamic and gamma-aminobutyric acids from the Recent sediment and of several amino acids from the Miocene rock may be accounted for in this way. It is clear that the organic material in the sediments was extensively destroyed during the heating experiments. The occurrence of phenol in the heated sediments testifies to this fact. It is suggested that the analytical procedure for the determination of amino acids in older sedimentary rocks be amended to include a heating step. Short-term heating with water in a bomb at temperatures around 150° C, prior to the acid refluxing step, may provide more information about the amino acid content of ancient rocks than simple HCl hydrolysis.

In general, the relative thermal stabilities determined for the amino acids in the sediments are not a great deal different from those found by Vallentyne(1964) using pure aqueous solutions of the individual compounds. However, differences are noted in the relative thermal stabilities of some amino compounds in the two sediments. Therefore, the path of thermal decomposition may differ in the two sediments. The relative thermal stabilities of glutamic acid, glycine, and phenylalanine change in the Recent sediment with heating time at temperatures around 240° C. At shorter heating intervals glycine and phenylalanine decline rapidly relative to valine: glutamic acid decreases gradually compared to valine. In samples heated for longer periods of time phenylalanine seems as stable as valine, while glutamic acid and glycine are more stable than valine. The relative thermal

stabilities for the Miocene amino acids are similar to those for the amino compounds from the Recent sediment heated for longer intervals. The initial decline of glutamic acid, glycine, and phenylalanine, relative to valine, in the Recent sediment may indicate that catalysis is affecting the thermal decomposition rates of these substances. The degradation rates of easily mobilized amino compounds would be altered the most by catalysis. Therefore, under the experimental conditions amino acids present in proteinaceous material might be expected to be more easily decomposed than those present in a more stable complex.

The apparent activation energy for the thermal alteration of alanine in the Recent sediment is much lower than the value determined for this substance in pure aqueous solutions. This may indicate that the decomposition rate is enhanced by catalysis or that alanine is altered by other chemical reactions. Both of these processes are expected to be more effective on dissolved compounds. Surface catalysis may be the most likely process affecting the decomposition rates of the amino acids in the Recent sediment.

Glycine, alanine, glutamic acid, valine, isoleucine, and leucine are the most abundant amino acids found in the heated sediments and humic acids. They are also among the more common amino acids found in geologic materials. Others have suggested that this is due to the relative thermal stability of these compounds. It is suggested here that it may also be due, in part, to their geologic availability at least for the rocks formed since Miocene time.

Except for the relative thermal stabilities the data obtained from laboratory studies on the thermal decomposition of amino

acids probably cannot be applied to sediments. This is not unexpected since sediments may contain a great many substances which may alter rates of reaction. The thermal decomposition rates for amino acids in a relatively simple system such as a carbonate shell may differ from those obtained from pure aqueous solutions. Another factor which complicates the sediment picture is the presence of relatively stable organic complexes which contain amino acids in measurable quantities. It seems evident that even the least stable amino compounds have survived since the Late Miocene in the Malaga mudstone. The present methods of attacking the organic substances in sediments are probably not capable of producing truly quantitative analyses for organic compounds. The methods probably destroy a portion of the substance sought. Therefore, it does not seem likely that amino acids in sediments will be useful for geothermometry except in a very gross way. The amino acids may be useful in areas where uniform sedimentary rocks have been subjected to rather steep temperature gradients for a relatively short time. It would be necessary to show that no subsequent organic contamination has occurred.

The data from the experiments indicate that the elimination of amino compounds from sediments during heating is rather complex. It is evident that several factors other than simple thermal decomposition are affecting the yields of amino acids obtained from the sediments.

Leucine and isoleucine react similarly to heating in the sediments. Their behavior in the two sediments is also much alike. The ratios of leucine to isoleucine are rather similar in heated and

unheated specimens of both rocks despite the fact that the sediments were subjected to relatively severe treatment. The leucine-isoleucine ratio is almost identical in the two unheated sediments although they are very different in age. This ratio may prove to be useful as an indicator of the source of amino acids in various organic geochemical reservoirs and also for the estimation of the fractionation of amino substances in fossils during diagenesis.

The increase of the carbon-nitrogen ratio of the Miocene sediment and humic acid may indicate that the processes of heating with water and acid hydrolysis serve to speed up the conversion of sedimentary organic matter to kerogen. Analogous processes, under milder conditions, acting over long spans of geologic time may be responsible for the formation of kerogen.

It is evident that the organic fraction of the Miocene rock has undergone diagenesis despite the fact that the sediment was apparently not subjected to great changes of temperature and pressure. No data are available on the present distribution of the organic constituents in the Miocene Malaga mudstone. Therefore, no attempt has been made to speculate on the means by which the organic constituents of the sediment have been altered.

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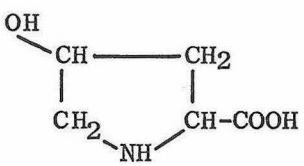
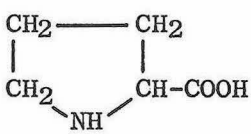
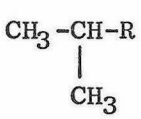
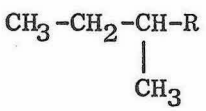
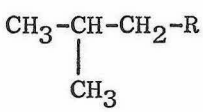
APPENDIX I

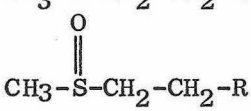
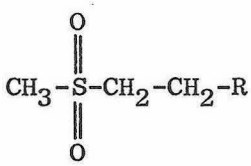
Formulae of the Amino Acids Discussed in this Paper

R = *CH(NH$_2$)-COOH * indicates alpha carbon atom

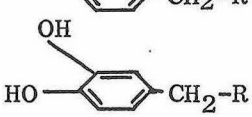
Name	Formula	M. W.
Acidic Amino Acids		
Aspartic Acid	HOOC-CH$_2$-R	133.1
Glutamic Acid	HOOC-CH$_2$-CH$_2$-R	147.1
Imino Acids		
Hydroxyproline		131.1
Proline		115.1
Hydroxyamino Acids		
Threonine	CH$_3$-CHOH-R	119.1
Serine	CH$_2$OH-R	105.1
Neutral Amino Acids		
Glycine	H-R	75.1
Alanine	CH$_3$-R	89.1
Valine		117.1
Isoleucine		131.2

Leucine		131.2
Beta-alanine	$NH_2-CH_2-CH_2-COOH$	89.1
Gamma-aminobutyric acid	$NH_2-CH_2-CH_2-CH_2-COOH$	103.2

Sulfur Amino Acids

Cystine	$(S-CH_2-R)_2$	240.3
Cysteic Acid	HSO_3CH_2-R	169.2
Methionine	$CH_3-S-CH_2-CH_2-R$	149.2
Methionine sulfoxide		165.2
Methionine sulfone		181.2

Aromatic Amino Acids

Tyrosine		181.2
Phenylalanine		165.2
Dihydroxyphenylalanine		197.2

It should be noted that many of these "amino acids" have not been recognized as being found in proteins. Some of them may be discovered as part of proteins in the future. Many of them are products of the chemical alteration of protein amino acids while others are known to be present in peptides or in the free form in living tissue.

APPENDIX II

ANALYTICAL PROCEDURES

Sample Preparation

After a sample was refluxed for twenty-four hours with 6 N HCl it was filtered and washed with boiling water. The filtrate was evaporated to dryness three times *in vacuo* to remove the hydrochloric acid. The residue in the evaporating flask was dissolved in distilled water. The pH was adjusted to 4 by the dropwise addition of concentrated ammonium hydroxide. The solution was then added to the top of a column of acid-washed Dowex 50 resin (Bio-Rad AG50W-X8) in the hydrogen form and the upper few centimeters were agitated with a clean glass rod. The solution was allowed to percolate slowly into the resin. The walls of the column were rinsed, and the washings were permitted to run into the resin bed. The resin was then rinsed by slowly percolating distilled water through it until the rinse water was free of chloride ions. The amino acids were eluted with a 2 N ammonium hydroxide solution. Most of the inorganic salts do not move on the column when eluted with 2 N ammonium hydroxide. The high content of alumina in the clays of the sediment and the amphoteric nature of aluminum hydroxide makes the complete separation of these substances difficult.

The eluent was evaporated to dryness *in vacuo* three times in order to expel ammonia from the sample. The aluminum hydroxide residue was leached with a hot 80% ethanol-water solution acidified to a pH of two with hydrochloric acid. The residue was filtered with a small glass

funnel having a very fine fritted filter. The ethanol-water solution was evaporated to dryness in a small porcelain evaporating dish at 35° C. The residue in the evaporating dish was dissolved and adjusted to an exact volume of one milliliter. The solution medium was 10% isopropanol-water acidified to a pH of one. The samples were stored under refrigeration to prevent their deterioration. The isopropanol was used to prevent bacterial contamination.

Amino Acid Analysis

Mixtures of amino acids are separated by means of ion exchange chromatography. In acidic solutions the following reaction takes place:

$$\underset{\text{neutral form}}{R\text{-}CH(NH_2)COOH} + H^+ \rightleftharpoons \underset{\text{cationic form}}{R\text{-}CH(NH_3^+)COOH}$$

therefore

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

and at a constant pH

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

In the presence of a cation exchange resin the latter expression gives the distribution coefficient for amino acids between the acidic solution and the resin. This distribution coefficient is different for each amino acid and is also dependent upon ionic strength and temperature.

The amino acids are fixed to the top of the ion exchange column quantitatively at a pH of 2.2. A 0.2 N Sodium citrate buffer at a pH of 3.28 is forced through the resin. The change in acidity causes the various amino acids to be released from the resin in different proportions depending upon their individual distribution

coefficients. Each successive increment of buffer which approaches a concentration band of amino acids has a lower concentration of amino acids than the preceding increment. The equilibrium is disturbed at that point and some of the amino acid cations convert to the neutral state and pass into solution. The least ionizable amino acids move more rapidly down the column since they are retarded less than those which are attracted to the column in greater proportion. This continuous adsorption and mobilization of the amino acids causes them to separate from each other. They are eventually resolved into buffer solutions of individual amino acids separated by buffer free of amino acids.

A second buffer with a pH of 4.25 follows the 3.28 buffer in order to separate several amino acids which move very slowly on the column at a pH of 3.28. The more basic amino acids require separation on a different shorter column using successive buffers of pH 4.26 and pH 5.28.

The separated amino acids pass through a capillary tube and are mixed with ninhydrin reagent which reacts with amino acids to form a blue compound and with imino acids (proline and hydroxyproline) to form a yellow compound.

The colored solution segments pass through a continuous flow colorimeter in which the intensity of color is monitored at wavelengths of 570 and 440 millimicrons. Three photometer assemblies consisting of flow cells, narrow wavelength band color filters, slits, and photovoltaic cells are used to monitor the color intensities. A constant light source is centrally located with respect to the photometer units.

Two units monitor the light intensity at 570 millimicrons using two different path-lengths. One path-length is approximately 40% of the other thereby permitting measurement of a wide range of amino acid concentrations in the same solution. The third photometer assembly monitors the intensity of light passing the 440 millimicron filter. A three channel recorder is driven by the output of the photovoltaic cells. The result is a continuous record of the changes in color intensity of the solution as it passes through the colorimeter. The amino acid analyzer used in this study is capable of separating amino acids in a fraction of the time required by other analyzers. The use of a finer-mesh resin and a higher flow rate has effectively lengthened the ion exchange column.

Sensitivity

Under ideal conditions 10^{-9} to 10^{-10} molar quantities of individual amino acids are sufficient to yield measurable peaks. It is essential that the sample solution be low in those organic and inorganic impurities which tend to impede separation. Very dilute amino acid mixtures, free of interfering impurities, may be added in sufficient quantity to yield absorption peaks large enough to measure with reasonable precision. The lower limit of measurement for the experiment is therefore controlled mainly by the level of contamination of the blanks and the concentration of interfering impurities.

The total range of sample concentrations measurable by ion exchange chromatography is vast inasmuch as the concentration and amount of solution added to the column can be controlled by the operator.

CALCULATION OF THE DATA

In general the identification of the amino acid peaks is not difficult. The position of a peak with respect to the elution front (R_f value) is essentially constant for the amino acids. It is not necessarily constant for the amino sugars and some amines. The positions of these substances are apparently affected markedly by slight variations in ionic strength and temperature. When necessary, peaks may also be identified by the height of the 440 millimicron peak relative to the 570 millimicron peak. This is essentially a constant for each amino acid.

Known amounts of a standard amino acid mixture are utilized to produce records for use as comparison standards. The area under a peak is a function of the quantity of the amino acid producing the peak. The area of a peak is integrated by multiplying the net height (in absorbance units) by the width (in dots) measured at the half-height. The dots are a measure of time. Constants obtained from the standard charts are used to estimate the quantity of amino acids in the unknown mixtures.

The H x W (height by width) constant usually refers to one micromole of an amino acid. To determine the concentration of an identified amino acid in an unknown, the H x W value of its peak is divided by the standard H x W constant for that particular amino acid. The quotient represents the number of micromoles of the amino acid in the aliquot of the unknown mixture added to the column. The aliquot applied to the column represents a given amount of sediment. The number of micromoles of the amino acid in the unknown aliquot is divided by the

weight of the sediment in order to get the concentration in micromoles per gram of sediment.

APPENDIX III

Table III-1, in this section, provides the heating data for the samples utilized in this experiment. Some samples were run for pre-determined periods of time. The maximum temperatures achieved are given as "to X°C." Only the total heating time is given for these samples. The sample runs intended for use in obtaining kinetic data were assumed to begin 5°C below the maximum temperature achieved during the run. The temperatures are given as ranges. The time noted as "Time of Run" covers the period during which the specimen was within the temperature range noted.

Table III-2 shows the yields of the amino acids as percentages of the yields from the unheated sediment samples. The data are plotted in Figures 2 through 8 and Figure 11. The data for tyrosine and phenylalanine, which are plotted in Figures 9 and 10, are in Tables 5-2 and 5-3 on pages 44 and 48. It was necessary to graph the yields directly for these two amino acids since they did not separate on the chromatograms of the unheated Miocene sediment.

Table III-1. LIST OF SAMPLES MEASURED IN HEATING EXPERIMENTS

Recent Marine Sediment

Sample Number	Temperature (° C)	Time of Run (min.)	Total Time (min.)
A-1		(unheated)	
A-2	to 160	--	88
A-3	157-162	266	354
A-4	to 190	--	120
A-5	195-200	359	479
A-6	to 235	--	193
A-7	238-244	1080	1269
A-8	to 240	--	161
A-9	240-245	174	367
A-10	240-245	1650	1850
A-11	to 303	--	124
A-12	306-311	386	510
A-13	to 198	--	240

Miocene Marine Sediment

B-1		(unheated)	
B-2	to 149	--	132
B-3	149-154	300	389
B-4	to 154	--	60
B-5	153-158	297	377
B-6	to 193	--	161
B-7	192-197	232	393

(continued on the next page)

Table III-1 (continued). LIST OF SAMPLES MEASURED IN HEATING EXPERIMENTS.

Sample Number	Temperature (° C)	Time of Run (min.)	Total Time (min.)
B-8	to 231	--	180
B-9	230-235	315	427
B-10	233-238	350	496
B-11	to 235	--	170
B-12	235-240	190	360
B-13	to 237	--	180
B-14	237-242	180	343
B-15	237-242	360	513
B-16	238-243	2400	2597
B-17	to 154	--	70
B-18	to 194	--	240
B-19	to 303	--	153
B-20	304-309	533	686
Humic Acid Extract From Recent Marine Sediment			
C-1		(unheated)	
C-2	to 201	--	240
C-3*	to 197	--	240
Humic Acid Extracts From Miocene Marine Sediment			
D-1		(unheated)	
D-2	to 193	--	240
D-3	241-245	508	686
D-4*	to 192	--	240

* Extracted with 6 N HCl before heating.

Table III-2. AMINO ACID CONCENTRATIONS IN THERMALLY TREATED SEDIMENTS AS PERCENT OF THE MEASURED CONCENTRATION IN UNHEATED SEDIMENTS.

Maximum temperatures between 235° and 245° C.

Sample Number	Max. Temp. (°C)	Total Time (min.)	Glutamic Acid	Proline	Glycine	Alanine
Recent Sediment						
A-1	(unheated)		100.0	100.0	100.0	100.0
A-8	240	161	29.9	36.5	17.3	39.0
A-6	235	193	25.7	35.4	5.41	27.2
A-9	245	367	11.2	16.4	2.62	11.9
A-7	244	1269	50.9	4.13	2.00	2.67
A-10	245	1850	37.7	2.30	0.88	0.93
Miocene Malaga Mudstone						
B-1	(unheated)		100.0	100.0	100.0	100.0
B-11	235	170	--	41.0	35.8	83.7
B-13	237	180	79.0	--	113.0	146.0
B-14	242	343	172.0	45.1	143.0	121.0
B-9	235	427	11.0	37.8	125.4	--
B-10	238	496	152.3	36.3	118.3	111.0
B-15	242	513	103.0	51.0	90.8	64.5
B-16	243	2597	66.5	28.6	28.9	20.1

Table III-2 (continued). AMINO ACID CONCENTRATIONS IN THERMALLY TREATED SEDIMENTS AS PERCENT OF THE MEASURED CONCENTRATION IN UNHEATED SEDIMENTS.
Maximum temperatures between 235° and 245°C.

Sample Number	Max. Temp. (°C)	Total Time (min.)	Valine	Iso-leucine	Leucine	Beta-alanine
Recent Sediment						
A-1	(unheated)		100.0	100.0	100.0	100.0
A-8	240	161	31.2	20.9	23.5	5.54
A-6	235	193	34.8	23.5	27.1	3.62
A-9	245	367	13.2	10.2	7.04	1.27
A-7	244	1269	2.07	9.78	0.25	0.63
A-10	245	1850	3.95	--	0.79	--
Miocene Malaga Mudstone						
B-1	(unheated)		100.0	100.0	100.0	100.0
B-11	235	170	55.5	43.3	43.9	1.87
B-13	237	180	81.0	55.8	56.1	5.39
B-14	242	343	51.4	35.6	30.7	3.90
B-9	235	427	47.1	23.9	28.4	2.67
B-10	238	496	45.3	26.6	25.5	2.02
B-15	242	513	39.5	12.3	14.1	2.78
B-16	243	2597	13.7	4.37	1.36	1.25