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# THE WASHINGTON ARCHAEOLOGIST

NEXT MEETING: Seattle Chapter - Wednesday, April 13, 1960 - 8:00 P.M.

MEETING PLACE: City Light's North Service Center at North 97th St. and Stone Avenue--2 blocks east of Aurora on North 97th St.

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"AMATEUR ARCHAEOLOGY IN GERMANY" was the subject of the March program of the Seattle Chapter. Doug Anderson, who spent a year in Germany working in museums in Hamburg and Munich, presented us with some of his firsthand observations regarding the relationship of the professional and amateur archaeologists there.

Archaeology in Germany takes in both the historical and the prehistorical. There seems to be a great awareness even among children of the importance of things pertaining to archaeology. School teachers are well informed and form a link between the laity and the museums. When something of significance is found it is usually reported to a teacher, who in turn relays the information to the museum in the area. The museum will send a professional to investigate and often, if there is a site to be excavated, the work will be done by amateurs under his direction. Following the dig, the professional will write the report and the artifacts will be given to the museum. However, if the owner of the land where the find has occurred does not wish to give up the material, he may keep it with the understanding that it stay all together and in his family. Mr. Anderson said many persons have fine collections which have been in their families for years.

At one archaeological meeting he attended in northern Germany, an amateur, as well as the professionals, gave a report of excavations he had carried out with an interpretation of the material found. The largest amateur group seems to be located in the southern part of the country.

Much publicity, including pictures, is given all archaeological finds in the German newspapers and other publications.

Following Mr. Anderson's presentation, the general membership discussed ways in which we might promote and make our society more effective. The April meeting of the Seattle Chapter will be devoted to a continuation of this discussion and to a general consideration of Society policy and plans for the coming season. The membership is urged to contribute suggestions, ideas, and questions.

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# AMINO ACID DATING

Project Report by Robert M. Downing

(Editor's Note: At the January, 1959, meeting of the Washington Archaeological Society, Seattle Chapter, Dr. Frederick P. Thieme discussed new developments in dating methods. As a direct result of these comments Mr. Downing chose Amino Acid Dating as his special science project for the Minnesota Public Schools. Dated bone samples for use in the experiments were provided by Dr. Douglas Osborne from the Mesa Verde. Other samples used were from Sites 45KT3, 45KT6, and Wakemap.)

## HISTORY OF AMINO ACID DATING

In 1810, W. H. Wollaston isolated the first amino acid, cystine. Later, 1819, Proust obtained leucine from wheat gluten, and Braconnot obtained glycine from gelatine in 1820. This was the beginning of modern protein chemistry.[1]

Since these early beginnings, over forty amino acids have been discovered. They are now known to be composed of organic acids in which one of the carbons is replaced with an amino or $NH_2$ group. Amino acids are most easily obtained by hydrolyzing protein with acids, alkalies, or enzymes. This destroys the colloidal characteristic of the protein and results in the crystalline amino acids.[2]

The amino acids are all quite similar in their basic characteristics. They are all somewhat soluble in water and organic solvents, and most of them have a density between 1.2 and 1.6 grams per milliliter. Finally, most of them melt and decompose around 300° C. and are composed mainly of carbon, hydrogen, oxygen, and nitrogen.

Currently, there is very little known about the behavior of amino acids when they are buried beneath the earth for considerable periods of years. However, it is known that amino acids will survive in bone and shell for long periods, and that many of the acids will disappear in time.

Work in the process has just begun, but if the amino acids decompose in a regular manner a process might well be worked out to detect the loss of these acids and to determine the relative age of the sample. Such a process could well be a valuable tool to the archaeologist.

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1 Richard J. Block, Outline of the Amino Acids and Proteins
2 Arlington Chemical Co., Proteins and Amino Acids

## PROCESS OF DERIVING AMINO ACIDS

Though amino acids have been obtained from proteins for many years, the process still remains quite complicated and time consuming. Also, great care must be exercised to prevent contamination of the sample since there are enough amino acids even in a finger print to cause serious discrepancies in the results.

There are three basic steps in the process of deriving and separating the amino acids. First, the substance must be hydrolyzed to break down the protein; second, the excess calcium salts must be removed; and finally, the solution of amino acids must be separated into the individual acids.

The first step is to break down the protein by hydrolyzation. The hydrolyzate usually used in amino acid work is hydrochloric or sulfuric acid. In addition to changing the protein to amino acids, the acid also breaks down the inorganic matter, so that the sample is entirely in solution after hydrolyzation. The solution containing amino acids also has an excess of the hydrolyzing acid which is removed by evaporation in the case of hydrochloric acid[3] or by the addition of barium hydroxide if sulfuric acid[4] was used as the hydrolyzate.

In either case, the excess water is removed by placing the sample in a dessicator until it is as dry as possible. The residue, containing the amino acids, is then redissolved in a 10% aquaeous solution of isopropyl alcohol and filtered to remove any insoluble salts.

The soluble salts in the solution can cause distortion on the chromatographs, so it is desirable to remove them, also. However, this step can be omitted with very little loss of quality. The ion exchange resin replaces the salt ions with hydrogen ions. To accomplish this, the resin is placed in a long tube, and the solution containing the amino acids is allowed to trickle through. The solution emerges from the resin relatively free from the inorganic salts. After this step, the solution of amino acids is ready to be separated into its component acids.

To be of any use as a dating process, the individual amino acids must be separated. This can be done most easily by paper chromatography, or, in a more difficult manner, by electrophoresis or by ion exchange. Since I used paper chromatography in my project, I will describe this process.

The type of paper chromatography which I employed is based on capillary action. A sample of the amino acids is deposited with a capillary tube in a line at one end of a piece of filter paper. The end with the line of amino acids is placed in a solvent, and the amino acids are carried along by the solvent as it rises by capillary action. The lightest amino acids are carried the farthest and deposited in a line, while the heaviest acids move the least. In this manner, the amino acids can be easily separated from each other.

Since the amino acids are basically colorless, they still cannot be detected, even though they are separated. Therefore, some indicator is needed to render them visible. The most common indicator is ninhydrin reagent, which reacts with the amino acids producing different shades of blue. The ninhydrin reaction takes place in two steps. First, the ninhydrin or triketo-hydrindene-hydrate removes the hydrogen from the amino acid with the formation of ammonia, aldehyde ($CH_3CHO$), and carbon dioxide. Second, the ninhydrin and aldehyde condense with

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3 Since hydrochloric acid is hydrogen chloride gas dissolved in water, the evaporation of the water leaves the hydrogen chloride gas and thereby removes the excess acid.

4 $H_2SO_4 + Ba(CH)_2 \longrightarrow 2H_2O + BaSO_4$ The acid is neutralized, and the $BaSO_4$ leaves the reaction as a precipitate.

the ammonia to yield a blue colored compound[5]. This reaction occurs with all naturally occurring amino acids except proline and hydroxoproline, which react differently to form a yellow compound. The ninhydrin is usually sprayed on the chromatograph and then allowed to dry, hotter temperatures speeding the reaction. After the development, the amino acids appear as varicolored bands which can be identified as specific acids.

The separated amino acids can now be used to establish a dating process. To do this, the chromatograph of a protein sample of unknown age is compared to the chromatograph of a modern one. Be determining the number of amino acids absent, the relative age of the sample can be estimated. Old samples of known age would first have to be used to calibrate the system to indicate what age corresponds to the absence of which amino acids. Once a system of this type has been calibrated, it should be possible to estimate the age of any protein containing substance over a considerable period of years.

Since bones are still well preserved even after remaining underground for long periods of time, they are the most likely source of amino acids for dating. However, other protein containing substances such as shell could also be used. Another possibility is wood or charcoal. However, these are less likely to prove useful, since wood usually decays after a short time, and the amino acids decompose above 300° C, a much lower temperature than that at which charcoal is formed. Calcification[6] of bone or wood would probably result in the loss of all protein. However, there is a possibility that there would be enough residual protein left to use the amino acids for dating purposes. If this were possible, the useful dating period of the process would be greatly extended.

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5 Carl L. A. Schmidt, Chemistry of the Amino Acids and Protein.

6 Calcification is the replacement of the structure of bone or shell with calcium compounds.

## EXPERIMENTATION IN AMINO ACID DATING

In my project on amino acid dating, the prime source of protein was bone. However, additional tests were made with egg albumin, shell, wood, and charcoal. The original tests were made with egg albumin because it has a very high concentration of protein. Also, there are relatively few salts remaining to cause distortion on the chromatographs. The process perfected on the albumin was later used on the more elusive acids in the other substances.

The process of deriving and separating the amino acids proved to be rather difficult due to the fact that so many steps were involved. Since the presence of the acids could not be detected until the paper chromatography was perfected, this step was developed first, and the steps antecedent to this, later.

Since I did not have any amino acids to work with in the beginning, I developed the paper chromatography technique by using a mixture of different colored inks. Through experimentation, I found that single dimensional chromatography using capillary action was the most effective. Two dimensional chromatography[7] resulted in greater dispersion, but the chromatographs were harder to interpret

and were less reliable. Single dimensional chromatography using gravity[8] worked faster, but resulted in a loss of definition.

I now knew that I could detect the amino acids if I could derive them from the albumin. The next step was to develop and perfect the other processes associated with their derivation. However, I had relatively little difficulty in deriving the amino acids now that I had perfected the chromatography needed to detect them. I discovered that It is usually better to omit the ion exchange step, since the salts caused only minor distortion on the chromatographs, whereas it was extremely easy to lose many of the amino acids in the ion exchange resin.

Having perfected the process on egg albumin, I graduated to bone as an amino acid source. A similar process was used on the bone as well as the other samples tested. The process which I have standardized for use in all of my tests is as follows:

1. 2 grams of the sample are hydrolyzed in 20 ml of 6N, HCl at 100° C. for 8 hours.

2. The sample is dried at 100° C., then placed in a dessicator for 24 hours or until all traces of hydrogen chloride gas are gone.

3. The sample is redissolved in 10 ml 10% isopropyl alcohol.

4. The sample is filtered to remove the humin and insoluble salts.

5. A three inch long line (narrow as possible) of the sample is placed one inch from the bottom of a 15-inch strip of Whatman No. 1 filter paper and allowed to dry.

6. A 1:1 solution of n-butyl and acetic acid (36%) is used as an irrigating solution in an air-tight glass tank.

7. The chromatograph is allowed to run at 20° C. until the solvent reaches a height of 14 inches. The chromatograph is then dried.

8. The chromatograph is sprayed with a 1% solution of ninhydrin and allowed to dry at 20° C. for at least 24 hours.

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7 Two dimensional chromatography is run in two directions at right angles to each other, using different solvents for each direction. This results in spots with greater dispersion than the lines produced by single dimension chromatography.

8 This uses the force of gravity to move the solvent and amino acids.

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RESULTS OF EXPERIMENTATION

The first samples tested were egg albumin. This was chosen because albumin is relatively free of salts and extremely high in protein content. When chromatographed, the hydrolyzate of egg albumin produced eleven distinct amino acids.

Following these tests of the system, I began working with samples of bone. The first thing necessary was to produce a standard sample of the amino acids contained in modern bone. To do this, I hydrolyzed 20 grams of beef hip bone. Using the process previously outlined, I obtained about 30 millileters of very concentrated amino acid solution. When chromatographed, this sample produced ten amino acids. All but three of the amino acids found in the albumin were present. A conspicuous addition was the yellow reacting proline and hydroxoproline. This sample of bone was then used as a comparison standard for all the subsequent tests.

The first samples of old bone experimented with were donated by the St. Paul Science Museum. These were unidentified animal bone taken from an archaeological site near Stillwater, Minnesota. The samples had been found in depths beneath the surface from three inches to four feet, in six inch levels. The age of these bones was from less than a hundred years for the bones nearest the surface to nearly one thousand years in the case of the deepest. When tested, all of these samples were found to still contain all of the ten amino acids in the standard sample. Even the oldest bones had lost none of the amino acids.

The next samples tested were human rib bones taken from an Indian burial mound near Brooten, Minnesota. These bones were located five feet beneath the surface and had an estimated age of approximately 2000 years. When the hydrolyzate of this bone was chromatographed, one of the amino acids was missing. This looked very promising, for it indicated that perhaps the amino acids did not take extremely long periods to decompose. Also, the loss of just one amino acid seemed significant for it might prove that the amino acids would decompose one at a time over a period of years.

Still older bones were now needed to show a loss of more amino acids. From the University of Minnesota, I obtained a sample of bison rib taken from a peat bed north of Minneapolis. This was found ten feet below the surface and had an estimated age of about 9000 years. When chromatographed, this sample of bone still contained all of the amino acids in the standard. This was unsuspected, for the 2000 year old bone had already lost one amino acid and it seemed likely to assume that one or more amino acids would be missing in the bison bone.

As a check against the results of the bison rib experiment, I obtained a sample of mammoth bone from the St. Paul Science Museum. This sample, taken from the femur of a mammoth found in northern Minnesota, also had an estimated age of 9000 years. The chromatograph of the hydrolyzate of this sample completely contrary to what had been expected. This sample had lost all of its amino acids.

The results obtained to this point did not follow the pattern expected for the behavior of amino acids. A 9000 year old sample had lost no amino acids, another 9000 year old sample had lost all of its amino acids, while a 2000 year old sample had lost one acid. One conclusion that could be drawn from this is that the amino acids did not decompose spontaneously as supposed, but instead other factors were at work in their decomposition, and their breakdown was a result of the varying external conditions they were exposed to. Apparently, conditions such as soil acidity, water, and mold could affect the decomposition of the amino acids. However, this assumption does not seem to be completely true, for it would seem reasonable that the extremely acid and wet conditions the bison bone was exposed to in the peat bed would be more destructive to the

amino acids than the more normal conditions that the mammoth bone was exposed to. Therefore, there would seem to be even other factors that decompose the amino acids. Perhaps even different kinds of bone decompose at different rates.

From the tests, it becomes apparent that a loss in the amino acid content of a bone would merely indicate that the bone had been exposed to different external conditions. It would have no bearing on the age, because severe conditions for a short period of time could just as easily destroy the amino acids as mild conditions for a long period of time.

This process could still have some use, however. If an archaeologist were interested in cheaply separating the bones of two cultures located in the same place, he might still be able to do this by the amino acid content of the bones. Since the bones could be assumed to have been exposed to the same conditions, the rate of decomposition of the acids should be the same and would depend only on the age of the bone. However, local conditions ground water, mold, and minerals could still cause unequal decomposition. Further tests made on samples taken from the same site will be needed to prove if this limited use of the dating process would be feasible.

All of the conclusions drawn so far are based on preliminary evidence and further experimentation will be needed to either completely prove or disprove the amino acid dating process.

BIBLIOGRAPHY

Arlington Chemical Company, Proteins and Amino Acids, Yonkers, New York, 1944.

Balston, J.N. & Talbot, B.C., A Guide to Filter Paper and Cellulose Powder Chromatography, H. Reeve Angel & Co., London, 1952.

Block, Richard J., Amino Acid Handbook, Charles C. Thomas, Springfield, Ill, 1956.

Block, Richard J., Outline of the Amino Acids and Proteins, Reinhold Publishing Corp., New York, 1952.

Block, Durrum, Zweig, A Manual of Paper Chromatography and Paper Electrophoresis, Academic Press, New York, 1958.

Brimley, R.C. & Barrett, F.C., Practical Chromatography, Reinhold Publishing Corp, New York, 1952.

Leaderer, Edgar & Michael, Chromatography, Elsevier Publishing Co., New York, 1957.

Nachod, Fredrick C., Ion Exchange: Theory and Application, Academic Press, New York, 1949.

Schmidt, Carl L.A., Chemistry of the Amino Acids and Proteins, Charles C. Thomas, Springfield, Ill., 1938.

Science, Amino Acids in Fossil Human Bone, October 18, 1957.

Science, Survival of Amino Acids in Marine Sediments, November 23, 1956.

Scientific American, Amino Acids in Shell, July 1956.

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## ECOLOGICAL INTERPRETATIONS OF ARCHAEOLOGICAL SITE DATA: MODOC ROCK SHELTER

(Abstract of paper read at the Twenty-fourth Annual Meeting of the Society for American Archaeology.)

Thousands of artifacts, animal bones and other evidence of human habitation have been recovered during the four seasons of archaeological excavation at the Modoc Rock Shelter by the Illinois State Museum field parties. These remains were studied to determine the ecological surroundings and the nature of adaptation of the Modoc inhabitants to their environment.

The basic contours of the bluff were formed by the torrents of the Valders Substage of the Wisconsin glaciation ca. 9000 B.C. The site was occupied shortly after these waters receded. The major occupations of the site spanned a period of 6000 years, from about 8000 B.C. to 2000 B.C. Sporadic use of the site took place as late as 1200 A.D. During this occupation the deposits of the site built up to a depth of over 27 feet above the bed rock floor.

The faunal data suggest that the major orientation of the Modoc hunters' quest for food was the river valley and marsh areas where fish, mollusks, waterfowl and deer were available. A few species indicate that they also hunted in the wooded uplands and prairie.

Analysis of the faunal remains suggests that the ecological setting remained much the same throughout the occupation of the site. The presence of one species of terrestrial snail, Allogonia profunda, in the lower levels of the site only indicates that the habitat may have been cooler and damper in those earlier periods than later. Otherwise, the same species were found throughout all levels of the site.

Analysis of the different categories of animals in relationship to depth found at the site indicated a changing preference of types hunted. In the earliest occupation extensive use was made of all species available. In the middle periods there was indication that specialization took place which utilized the types of animals most abundant in the immediate area, viz., fish, mussels and aquatic snails. During the last periods of Archaic occupation, inhabitants became specialized hunters of primarily deer and waterfowl.

A similar analysis of artifact categories demonstrated that in the early and middle periods of the site both hunting and domestic activities were represented in the assemblage, whereas in the later Archaic period hunting tools dominated. On the basis of these interpretations the ecological adaptation of the Modoc peoples through time was divided into three sequent phases:

Phase I: The Initial Occupation; 8000-6000 B.C.; these people were scavengers who utilized all available resources.

Phase II: Localized adaptation, 6000-3500 B.C.; the subsistence changed to exploit more fully certain food resources available in the immediate area.

Phase III: Specialized adaptation, 3500-2000 B.C., the hunting of deer and waterfowl, dominated and the occupation of the site changed from a general habitation to a specialized hunting camp. Other activities were probably carried on in other specialized camps.

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## A TENTATIVE CULTURAL SEQUENCE FOR THE LOWER SNAKE RIVER REGION

(Abstract of paper read at the Twenty-fourth Annual Meeting of the Society for American Archaeology.)

Sufficient work has been done at sites located in the lower Snake River valley to make an attempt at outlining the sequence of regional cultural change and development justifiable and necessary.

Broadly, the period since late Pleistocene times can be divided into five major periods: Paleo-Indian, Transitional, Developmental Snake River, Snake River and Historic. In addition to sketching the characteristics of each of these periods, it is a purpose of this paper to attempt to relate the lower Snake River region wo the "Plateau Culture Area." I believe it can be demonstrated that the lower Snake River region is a distinctive "Plateau" sub-area with continuing strong cultural ties with the Great Basin to the south. Perhaps a more correct way to phrase this relationship is to state that the lower Snake River region has been a strong participant in what Jennings and others have referred to as the "Desert culture." An example of the sub-areal characteristic of the lower Snake region is the fact that such late cultural elaboration of the adjacent middle-Columbia region as the distinctive carving in bone and stone, and the elaborate cremation complex, never penetrated the valley of the Snake River above its mouth.

Additional work must be done to provide a more complete inventory of cultural materials representing these various cultural periods, to indicate subdivisions within these broad periods and to demonstrate more fully the nature and extent of cultural relationships.

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