13 at 133 cm and sample 8-15 at 89 cm. Previous K–Ar data (*9*) would suggest an age of about 15 million years for this part of the Miocene, which is somewhat older than the 11.4 and 12.3 million year ages which I have determined for samples 8-13 and 8-15, respectively. Considering the problems of correlating this core with previously dated stratigraphic sections on land, as well as the errors of the K–Ar measurements, this agreement is considered satisfactory. The sedimentation rate for this predominately calcareous-siliceous ooze between sample 9-1 at 46 cm and sample 8-15 at 89 cm (42 to 132 meters below the sediment surface) is 1.1 cm/10^3 years. This compares with an ionium-thorium rate of 8 mm/10^3 years, determined on a gravity core, 230 centimeters long, from the same area (*5*).

The use of acidic glasses to record time in marine sediments was extended to a series of four cores taken in the waters off Central America (Fig. 1). Cores from this area commonly contain layers of volcanic glass, many of which are remarkably pure bands of acidic glass shards, perhaps similar in origin and type to those described by Worzel (*10*). Here the assumptions of rapid deposition and exclusion of detritus are most probably fulfilled, and there is little reason to suspect an argon loss by diffusion during this time range (less than 10^6 years). The rates of accumulation for these cores range from 3.0 mm/10^3 years to 5.0 mm/10^3 years. Cores Zap 3P, Zap 6P, and Zap 8P each contain an ash layer which gives an age that agrees within the limits of error, so that these layers may have originated from the same volcanic event.

In summary, this initial study indicates that K–Ar geochronology can be applied to the volcanic phases of pelagic sediments and that it is a means of dating the time of deposition. The long half-life makes it applicable to the entire Tertiary, and careful work may permit its application to materials less than 100,000 years in age. The limiting feature of the method is its reliance upon volcanic contributions. However, the abundance of submarine volcanoes, particularly in the Pacific (*11*), and the common occurrence of volcanic contributions in pelagic sediments (*12*) suggest that it has wide application.

JACK R. DYMOND
University of California, La Jolla

References and Notes

1. F. F. Koczy, in *Progr. Oceanogr.* **3**, 155 (1965).
2. E. D. Goldberg, M. Koide, J. J. Griffin, M. N. A. Peterson, in *Isotopic and Cosmic Chemistry*, H. Craig, S. L. Miller, G. J. Wasserburg, Eds. (North-Holland, Amsterdam, 1963), p. 226.
3. J. A. Cooper, *Geochim. Cosmochim. Acta* **27**, 525 (1963).
4. R. Bieri, in preparation.
5. E. D. Goldberg and M. Koide, *Geochim. Cosmochim. Acta* **26**, 417 (1962).
6. W. R. Riedel and B. M. Funnell, *Quart. J. Geol. Soc. London* **120**, 305 (1964).
7. E. Martini and M. N. Bramlette, *J. Paleontol.* **37**, 845 (1963).
8. M. N. Bramlette, personal communication, February 1966.
9. B. M. Funnell, in *The Phanerozoic Time Scale*, W. B. Harland, A. G. Smith, B. Wilcox, Eds. (Geological Society, London, 1964), p. 179.
10. J. L. Worzel, *Proc. Nat. Acad. Sci. U.S.* **45**, 349 (1959).
11. H. W. Menard, *Marine Geology of the Pacific* (McGraw-Hill, New York, 1964), p. 87.
12. M. N. A. Peterson and E. D. Goldberg, *J. Geophys. Res.* **67**, 3477 (1962).
13. Argon analyses were supported by AEC grant AT(11-1)34, project 84 and potassium analyses were supported by NSF grant GP-489. I thank R. Bieri, E. D. Goldberg, M. Koide, M. N. A. Peterson, Scripps Institution of Oceanography, for interest and help. I acknowledge a Kennecott Copper Corporation fellowship during this research.

28 February 1966

N-Acetylhistidine Isolated from Frog Heart

Abstract. *A diazo-positive compound was found in an extract of frog heart. This substance showed a negative ninhydrin reaction and had on a paper chromatogram developed with a phenol–hydrochloric acid solvent system a higher* R_F *than carnosine. The substance, isolated by chromatography, was identified as* N-*acetyl*-L-*histidine.*

Carnosine is contained in heart tissue as well as the skeletal muscle of various vertebrates (*1*). Paper chromatograms, developed by 80 percent phenol in an atmosphere of HCl (*2*), of hot-water extracts of animal hearts, indicated the presence of two diazo-reacting substances, histidine and carnosine.

In frog heart tissue we discovered another unique diazo-positive component of greater mobility than histidine or carnosine (R_F value: histidine 0.24, carnosine 0.47, and the unknown 0.74) on the chromatogram. The component could be isolated from many ninhydrin-reacting contaminants by rechromatography with (i) a mixture of *n*-butanol, acetic acid, and water (120:30:50); (ii) a mixture of *n*-propanol and 0.2*N* NH_4OH (3 : 1), and (iii) a mixture of *n*-propanol and 1*N* acetic acid (3 : 1). This newly isolated substance was ninhydrin-negative.

After digestion for 2 hours in 6*N* HCl at 100°C, a ninhydrin- and diazo-positive spot corresponding to histidine was detected on the paper chromatograms, an indication of an *N*-substituted amino acid.

Paper electrophoresis with a barbiturate buffer (*p*H 8.6, 0.075*M*) or a pyridine-acetate buffer (*p*H 4.0, 0.1*M*) indicated that the substance was more acid than neutral amino acids.

The hydrolyzate was analyzed for acyl groups by a chromatographic technique (*3*), and the analysis indicated the presence of either an acetyl or a formyl group.

Acylhistidines including succinyl-, propionyl-, acetyl- and formylhistidines were synthesized, and the substance was compared with these standards. In solvent systems of 80 percent phenol in an atmosphere of HCl; a mixture of *n*-butanol, acetic acid, and water; a mixture of *n*-propanol and 1*N* acetic acid; or others, the R_F values of the unknown component coincided well with that of acetylhistidine. However, in a developing mixture of lutidine and collidine the substance had a lower R_F value than acetylhistidine or formylhistidine. The substance mixed with authentic acetylhistidine yielded two different spots in this solvent system. Unequivocal identification of the unknown component was therefore not possible.

At this stage, large quantities of bullfrog (*Rana catesbeiana*) hearts became available and were stored in 95 percent ethanol. The substance was easily extracted from about 300 frog hearts with 1 liter of ethanol. The ethanol was evaporated and the residue was treated with ether, to remove fatty impurities. The residue was then dialyzed against distilled water for about 12 hours and the outside fluid was concentrated at reduced pressure.

The water-soluble residue was chromatographed (3.0- by 140-cm column) with a pyridine-acetate buffer (*p*H 3.1, 0.1*N*) on Dowex 50-x8 (300 to 400 mesh, buffered at *p*H 3.1). Each 10 ml of the effluent was collected mechanically; fractions that were diazo-positive and ninhydrin-negative were pooled and evaporated at reduced pressure. The product still contained a trace amount of ninhydrin-positive impurities that could not be removed by column chromatography on Dowex-1.

Preparative paper chromatography

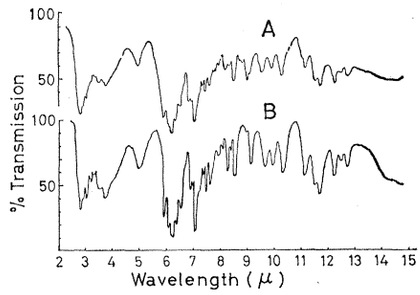

Fig. 1. Infrared spectra in KBr of (*A*) crystallized substance from frog heart extract and (*B*) a sample of synthetic *N*-acetyl-L-histidine monohydrate.

was carried out on wide sheets of Toyoroshi No. 50 paper with a mixture of pyridine, *n*-butanol, and water (1:1:1). The water eluate of corresponding sections of paper was concentrated and dried over calcium chloride in a desiccator, about 30 mg of crude crystals of the unknown component being obtained. This substance was ninhydrin-negative and its R_F value coincided exactly with that of synthetic acetylhistidine in all the aforementioned solvent systems.

Dinitrophenyl (DNP) derivatives of this substance and synthetic acetylhistidine gave colorless, diazo- and ninhydrin-negative spots of the same R_F value; the spots were detectable by ultraviolet absorption. The properties of the derivative were similar to those of imidazole-DNP-*N*-acetylhistidine. After hydrolysis in HCl these DNP- derivatives shifted to a lower R_F corresponding to that of imidazole-mono-DNP-histidine, which revealed a colorless, diazo-positive (yellow), ninhydrin-positive, and ultraviolet-absorbing spot.

The purified substance was identical with synthetic *N*-acetyl-L-histidine monohydrate (*4*) by infrared spectroscopy (Fig. 1), elementary analysis, and measurements of melting point and specific rotation.

The isolation of a simple acetylated amino acid from animal tissues has been reported heretofore only in two instances, acetylaspartic acid (*5*) from brain and acetylglutamic acid (*6*) from liver. They are both characterized by acetyl derivatives of acidic amino acid. The presence of acetylhistidine in frog heart is unique and is the first isolation of an acetylated basic amino acid.

Newt hearts also contain acetylhistidine. Thus acetylhistidine may be a common component of amphibian hearts.

YOSHIO KURODA
TATSUO IKOMA
Kyuden Institute of Health, Kyuden Hospital, Kashii Fukuoka, Japan

References

1. Y. Kuroda, S. Yamauchi, H. Fukae, *Seikagaku* **36**, 268 (1964).
2. T. Wood, *J. Sci. Food Agric.* **3**, 196 (1956).
3. A. R. Jones, E. J. Dowling, W. J. Skraba, *Anal. Chem.* **25**, 394 (1953).
4. M. Bergmann, L. Zervas, *Biochem. Z.* **203**, 280 (1928); R. Marshall, S. M. Birnbaum, J. P. Greenstein, *J. Amer. Chem. Soc.* **78**, 4636 (1956).
5. H. H. Tallan, S. Moore, W. H. Stein, *J. Biol. Chem.* **219**, 257 (1956).
6. L. M. Hall, R. L. Metzenberg, P. P. Cohen, *ibid.* **230**, 1013 (1958).

28 February 1966

Control of Myocardial Contraction: The Sensitivity of Cardiac Actomyosin to Calcium Ion

Abstract. *The control by calcium ion of the adenosine triphosphatase activity of cardiac actomyosin is similar to that of white skeletal actomyosin. This finding indicates that the slower contraction and relaxation of heart muscle do not reflect different levels to which free calcium ion concentration around the myofibrils must be adjusted during contraction and relaxation and suggests a mechanism whereby myocardial contractility may be regulated.*

It is possible that the slower contraction cycle in cardiac muscle, when compared with that of white skeletal muscle, is due to different concentrations of free Ca^{++} needed for activation of the myofilaments. Release of Ca^{++} by the sarcoplasmic reticulum of both cardiac and skeletal muscle now appears to constitute an essential step during excitation-contraction coupling, in which an action potential at the muscle cell surface initiates shortening and tension development by the myofilaments. Evidence that the sarcoplasmic reticulum is able to take up and store Ca^{++} has been obtained in studies of the intact muscle (*1*) and subcellular fractions isolated from muscle homogenates (*2*). Furthermore, both the adenosine triphosphatase activity and syneresis of isolated skeletal myofibrils and actomyosin are markedly inhibited by reduction of the Ca^{++} concentration to levels that can be achieved by the sarcoplasmic reticulum in the intact muscle (see *3*).

More recently it has been recognized that a tropomyosin-containing protein complex is necessary to mediate the inhibitory action of Ca^{++}-binding agents such as 1,2-bis-(-2-dicarboxymethylaminoethoxy) ethane (EGTA) (*4–6*) because reconstituted actomyosin made from highly purified actin and myosin is not responsive to these physiological changes in Ca^{++} concentration (*4*). In the present study, Ca^{++}-sensitive reconstituted actomyosins made with actin containing this tropomyosin complex and either skeletal or cardiac myosin have been compared.

Dog cardiac and rabbit white skeletal myosins were prepared by a method that minimizes contamination with actomyosin (*7*). Tropomyosin-containing "Straub" actins were prepared by extracting the acetone-dried muscle powders at room temperature (*6*) and reconstituted actomyosins were made by adding three parts, by weight, of myosin to one part of tropomyosin-containing F-actin (*6*). We prepared Ca^{++}-buffers by combining EGTA and CaEGTA (*3*), using the dissociation constants for the CaEGTA complex provided by Chaberek and Martell (*8*). Adenosine triphosphatase activities were calculated from the initial rates of liberation of inorganic phosphate, the latter determined by the method of Taussky and Shore (*9*). Traces of calcium were removed from adenosine triphosphate by treatment with Dowex 50.

Comparison of the Mg^{++}-activated adenosine triphosphatase activities of the reconstituted actomyosins prepared from the same tropomyosin-containing white skeletal actin and either dog cardiac myosin or rabbit white skeletal myosin revealed differences similar to those observed when these myosins were combined with tropomyosin-free actin (*7*).

At the KCl concentration of 0.08*M* used in these experiments, the actomyosin made with white skeletal myosin was eight to ten times as active as that made with cardiac myosin when the Ca^{++} concentration was greater than $10^{-4}M$. At this level of Ca^{++}, maximal activation of myosin adenosine triphosphatase by actin was seen. Although the adenosine tri-