Electron microscopy revealed striking similarities between the mineral deposits recovered from the bags and those obtained previously from developing human dental calculus (3). In both types of specimens areas were found in which mineralization occurred adjacent to and within the organisms (Fig. 4). Clusters of crystals were also found, which undoubtedly correspond to the refractile bodies seen optically.

The studies reported here indicate that a variety of bacterial implants will undergo calcification. While the presence of hydroxyapatite in biological specimens is usually but not always associated with higher forms (4), it is clear that even bacteria will mineralize with hydroxyapatite under proper conditions. Although the mechanism is at present unknown, the accelerated mineralization of nonviable organisms suggests that metabolic processes of the bacteria are not essential for mineralization and may indeed retard it.

A. A. RIZZO, G. R. MARTIN,\* D. B. SCOTT, S. E. MERGENHAGEN National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland

#### References

- S. E. Mergenhagen, G. R. Martin, A. A. Rizzo, D. N. Wright, D. B. Scott, Biochim. et Biophys. Acta 43, 563 (1960).
   M. N. Gilmour, A. H. Howell, Jr., B. G. Bibby, Bacteriol. Rev. 25, 131 (1961).
   H. A. Zander, S. P. Hazen, D. B. Scott, Proc. Soc. Exptl. Biol. Med. 103, 257 (1960); F. Gonzales and R. F. Sogennages, Science
- F. Gonzales and R. F. Sognnaes, Science
- F. Gonzales and R. F. Sognnaes, Science 131, 156 (1960).
  F. G. E. Pautard, in Calcification in Biologi-cal Systems, R. F. Sognnaes, Ed. (AAAS, Washington, D.C., 1960), p. 1.
  \* Research associate, American Dental Associa-tion
- tion.
- 24 August 1961

## **Contribution of E-Amino Groups to Ninhydrin Color Production in Proteins**

Abstract. The ninhydrin color given by model compounds and by characterized proteins shows a consistent contribution from the  $\epsilon$ -amino group of lysine of about 67 percent of that of an  $\alpha$ -amino group, except in free lysine or in N-terminal lysine, where the  $\epsilon$ -amino group makes a small contribution (7 to 10 percent) to the total color. This information can be applied to structure determination of Ne peptides of lysine.

In view of the current interest in atypical linkages in proteins (1, 2), it seems pertinent to point out that the ninhydrin color reaction can be applied to distinguish free  $\epsilon$ -amino and free  $\alpha$ -amino groups. The color developed

9 FEBRUARY 1962

Table 1. Ninhydrin color yields from compounds with known contents of  $\epsilon$ -amino and  $\alpha$ -amino groups.

Compound	Leucine equivalents per mole	No. of ε-amino groups	No. of $\alpha$ -amino groups	Leucine equivalents per $\epsilon$ -amino group*
Leucine	1†		1	
Lysine	1.10	1	1	0.10
Lysylglycine	1.08	1	1	0.08
N <sup>e</sup> -Carbobenzoxylysine	1		1	
N <sup>c</sup> -Acetyl lysine	1		1	
ε-Aminocaproic acid	0.67	1		0.67
N <sup>α</sup> -Tosyl-lysine benzyl ester	0.67	1		0.67
Methemoglobin (human) (8)	34.3	43	4	0.70
Conalbumin (9, 10)	37.2	60	1	0.60
Lysozyme (10, 11)	4.35‡	6	l(lys)	0,67
Ribonuclease (12)	7.70†	10	1 (lys)	0.74
Insulin (13)	2.67	1	2	0.67
N <sup><math>\epsilon</math></sup> -(Glycyl- $\alpha$ -glutamyl)lysine (1)	2.00		2	
Hydrolyzate of above (1)	2.87§	1	3	-0.13

\* Except for lysozyme and ribonuclease, yields (column 5) are given in leucine equivalents per mole (column 2) minus the number of  $\alpha$ -amino groups (column 4), the remainder being divided by the number of e-amino groups (column 3). For lysozyme and ribonuclease, the divisor is one less than the number of e-amino groups.  $^{+}$  By Moore and Stein method, as well as Troll-Cannan method.  $^{+}$  Tallan and Stein (14) found .70 leucine eq per amino group, or 4.9 leucine eq per mole for lysozyme.  $^{+}$  The color increase on hydrolysis of the peptide was less than expected, the theoretical value being 3.10 value being 3.10.

from  $\epsilon$ -amino groups is similar in a variety of compounds studied. When the Troll-Cannan method (3) is used, the molar extinction is 65 to 70 percent of that of leucine. An exception occurs when the  $\epsilon$ -amino and  $\alpha$ -amino groups are in the same residue [free (3) or Nterminal lysine]. Here the total color is only 107 to 110 percent of that of leucine; the apparent  $\epsilon$ -amino contribution is only 7 to 10 percent of that attributable to the free  $\alpha$ -amino group (4).

These conclusions were drawn from studies of model compounds and of certain purified proteins (5) for which complete analytical data are available.

The details of the Troll-Cannan method are as follows: a sample containing 0.05 to 5  $\mu$ mole of amino groups in 0.4 ml of water is heated in 1 ml of 80 percent phenol and 1 ml of 0.0002M KCN in pyridine. One-fifth of a milliliter of 5 percent ninhydrin in alcohol is added, and the mixture is boiled 3 to 5 minutes. The solution is cooled, diluted to 10 ml with 60 percent alcohol, and the optical density is read at 570  $m_{\mu}$ . The method, when applied to proteins, may give a white precipitate, but this has no apparent effect on color development in the supernatant.

Table 1 shows the results obtained from a variety of compounds studied.

To obtain the molar extinction per  $\epsilon$ -amino group, the extinction calculated for the  $\alpha$ -amino groups was subtracted from the total extinction per molecule, and the difference was divided by the number of  $\epsilon$ -amino groups. In the case of ribonuclease, the total color was attributed to one  $\alpha$ - and nine  $\epsilon$ -groups, since the terminal lysine contribution

should be that of one  $\alpha$ -amino group only, as found for lysine (3) and for lysylglycine. Similarly, in the case of lysozyme, which also possesses an Nterminal lysyl residue, the total color obtained was attributed to one  $\alpha$ - and five  $\epsilon$ -amino groups. The results are expressed in leucine color equivalents, as the absolute value for the molar extinction of leucine varies with the method used. It is noteworthy that, when the Moore and Stein method (6)was used on ribonuclease, the same results were obtained.

These observations have already proved helpful in assigning a structure to an  $\epsilon$ -amino linked peptide of lysine (Table 1) which was isolated from a partial hydrolysate of collagen (1, 7).

E. SLOBODIAN\*

G. MECHANIC<sup>†</sup> M. LEVY

Departments of Biochemistry, New York University Colleges of Medicine and Dentistry, New York

#### **References** and Notes

- 1. G. Mechanic and M. Levy, J. Am. Chem. Soc. 81, 1889 (1959).
- G. Mechanic and M. Levy, J. Am. Chem. Soc. 81, 1889 (1959).
   R. Schweet, Federation Proc. 15, 350 (1956); J. H. Bowes and R. H. Kenten, Biochem. J. 43, 358 (1948); A. Veis and J. Cohen, J. Am. Chem. Soc. 78, 6238 (1956); J. J. Betheil and P. M. Gallup, Biochim. et Biophys. Acta 45, 598 (1960).
   W. Troll and R. K. Cannan, J. Biol. Chem. 200, 633 (1953).
- 200, 803 (1953).4. Perhaps some of the lysine is converted to
- compounds other than the typical blue color of the ninhydrin reaction. It is not possible state which of the two amino gro puld actually be involved in a side groups
- to state which of the two amine groups would actually be involved in a side reaction. Therefore, the assignment made is a matter of convenience.
  5. We thank Dr. R. C. Warner for samples of crystalline lysozyme and conalbumin and Dr. J. Dancis for a sample of crystalline human methemoglobin. Insulin was obtained from Armour Co. from Armour Co.; ribonuclease was ob-tained from Worthington Biochemical Corp.

- 6. S. Moore and W. H. Stein, J. Biol. Chem. 176, 367 (1948).
- This work was aided by grants from the U.S. Public Health Service (RG-4902) and the National Science Foundation, and by an American Cancer Society institutional grant to New York University Medical Cenreport should ter. Enquiries concerning this be addressed to M. Levy, New York Uni-
- be addressed to M. Levy, New York Oniversity College of Dentistry, New York.
  R. R. Porter and F. Sanger, Biochem J.
  42, 287 (1948); H. S. Rhinesmith, W. A. Schroeder, L. Pauling, J. Am. Chem. Soc. 8. Schroeder, L. Pauling, J. Am. 79, 4682 (1957). 9. H. Fraenkel-Conrat and R.
- R. Porter. Biochim. et Biophys. Acta 9, 557 (1952).
  10. J. C. Lewis, N. S. Snell, D. J. Hirschmann, H. Fraenkel-Conrat, J. Biol. Chem. 186,
- 23 (1950). Schroeder, J. Am. Chem. Soc. 74, 11. W.
- W. A. Sch 5118 (1952)
- 5118 (1952)
  12. C. H. W. Hirs, S. Moore, W. H. Stein, J. Biol. Chem. 211, 941 (1954); C. B. Anfinsen, R. R. Redfield, W. L. Choate, J. Page, W. R. Caroll, *ibid.* 207, 201 (1954).
  13. F. Sanger, Biochem. J. 39, 507 (1955).
  14. H. H. Tallan and W. H. Stein, J. Biol. Chem. 200, 507 (1953).
  \* Present address: Department of Radiology, New York University Medical Center New
- New York University Medical Center, New York.
- Present address: Orthopedic Research Lab-Massachusetts General oratories, Hospital. Boston.

18 August 1961

# **Dimensional Fabric and Ice Flow**, Precambrian (Huronian) Glaciation

Abstract The Huronian Cobalt tillite (Gowganda formation) exhibits a preferred orientation of dimensional embedded elongate rock fragments. The orientation is believed to be due to ice motion. Data from four localities show an alignment in about a north-south direction. Data are inadequate to locate center of ice dispersal.

That till commonly exhibits a preferred orientation of the stones embedded in it is now rather generally known. The fabric exhibited by elongate stones has been commonly used to deduce the direction of ice flow and is a readily mappable directional property. To date, success in mapping till fabric has been confined to the Pleistocene deposits.

It occurred to me that this method might also be applied to the tillites of the Precambrian Cobalt series in Ontario. These glacial formations are little altered and little deformed and are well exposed over an area in excess of 8000 square miles. Inasmuch as the tillite appears to have a dimensional fabric (1, p. 492), I made a number of fabric analyses this past summer.

A suitable outcrop, having a glacially polished (by Pleistocene ice) nearly horizontal surface, was chosen. A square yard was marked out and the azimuths of the apparent long axes of all fragments having a length-to-breadth ratio greater than 2 were measured. In general, such restrictions provided about 50 measurable stones. The fragments ranged in length from a few millimeters to several centimeters. The measured azimuths were plotted in 30deg classes as circular histograms (Fig. 1). In several cases a second square yard was marked out and an additional 50 orientations were measured and plotted.

As is apparent from inspection of Fig. 1, a preferred orientation was found at all four localities. As is apparent also, the general orientation is roughly the same at all places, even though the observation points are many miles apart. The results are similar to those obtained by Dreimanis from his study of the dimensional fabric in an oriented hand specimen.

Several problems arise in making any interpretation of the diagrams. The long dimension measured is the ap-



Fig. 1. Pebble orientation in Precambrian Cobalt tillite (Gowganda formation). Bruce Mines: lot 2, Con. VI. Keating Township, Ontario; Iron Bridge: highway 17, 4 miles east of Iron Bridge, Ontario; Latchford (N): highway 11, 2.2 miles south of Latchford, Ontario; Latchford (S): highway 11, 4.3 miles south of Latchford.

parent long axis, and not necessarily the truly longest dimension of the fragment. One can imagine shapes and orientations which might give misleading azimuths. The consistency of the results suggest that such anomalous azimuths are relatively unimportant.

It is possible, of course, that the fabric is imposed by deformation or is produced in some other way than by ice flow. In most places it is not possible to determine the dip of the bed involved, owing to the massive nature of the tillite. In general, however, dips in the areas studied are very low and the glacial pavements studied may be presumed to be parallel to the bedding. The near-horizontal position of the strata over large areas makes a deformational fabric improbable. The similarity of orientation found in the widely separated localities and the absence of any relation between the fabric and the known tectonic structures renders a tectonic origin of the fabric unlikely.

If the fabric is induced by ice flow, as seems probable, we do not know whether the ice moved from south to north or vice versa. It is of interest, however, that the direction of movement suggested by the fabric is essentially the same as that shown by crossbedding in the overlying Lorrain quartzite, which in the Bruce Mines area indicates flow from northwest to southeast and in the Latchford area demonstrates a flow from north or a little east of north to the south. Did the Cobalt ice also move from north to south down the same slope? Or did the ice move upslope over long distances as did the Pleistocene glaciers of North America?

We can probably safely conclude that the pattern of ice flow in Huronian glacial times can be worked out from a study of the dimensional fabric exhibited by the stones embedded in the tillites and that it may be possible to establish a center of ice dispersal in these times as it is for the glacial deposits of the Pleistocene.

F. J. Pettijohn Department of Geology, Johns Hopkins University, Baltimore, Maryland

### Reference

Dreimanis, J. Sediment. Petrol. 29, 459 (1959).

12 September 1961