Calcium Carbonate Concretions Formed by the Decomposition of Organic Matter

Abstract. *Bacterial decomposition of butterfish and smelts in small sealed jars containing seawater and other solutions, for periods ranging from 65 to 205 days, results in a large increase in concentrations of dissolved bicarbonate, carbonate, and ammonia (plus volatile amines). Accompanying this is a rise in* pH *and the precipitation of* Ca^{++} *ion from solution. The* Ca^{++} *is not precipitated as* $CaCO_3$ *but instead as a mixture of calcium fatty acid salts or soaps with from 14 to 18 carbon atoms. This can be explained by the thermodynamic instability of* $CaCO_3$ *relative to Ca soaps in the presence of excess free fatty acid. It is suggested that some ancient* $CaCO_3$ *concretions, especially those enclosing fossils of soft-bodied organisms, may have formed rapidly after death in the form of natural Ca soap (adipocere) which was later converted to* $CaCO_3$.

Well-preserved fossils of ancient organisms, including carbonized and mineral replacements of soft tissues, are often found within calcium carbonate concretions. Association of concretions with fossils has led to the speculation that bacterial decay of the soft parts of the original organism was an important cause of precipitation of the $CaCO_3$ (*1*). Such a theory is problematical in that field measurements of natural waters and sediments indicate that organic decay generally results in a drop in *p*H and the production of carbonic and other acids which, if anything, should dissolve $CaCO_3$. However, measurements of natural *p*H, due to diffusion and mixing processes, are space- and time-averaged values, and the microenvironment about any given organism at any given instant, especially the time immediately after death, is not generally sampled. Proteinaceous organisms, upon decay, not only release carbonic and other acids but also give off basic substances such as ammonia and amines. At different stages of bacterial attack, depending upon various ecological factors, such as *p*H and the content of dissolved oxygen, the relative rates of release of acid and base can vary. The common observation that organic matter in modern sediments has a considerably higher ratio of carbon to nitrogen than the original organisms which contribute the organic matter (*2*) suggests that loss of nitrogen is most rapid during the earliest stages of decay. This is shown further by experimental studies of the decomposition of plankton (*3*) and fish (*4*). If the nitrogen is lost in the form of ammonia and other bases, it is possible that initial decomposition of organic matter may result in a rise in *p*H instead of a decrease. If this is true, rapid formation of a base may enable the precipitation of $CaCO_3$:

$$NH_3 + Ca^{++} + HCO_3^- \rightarrow CaCO_3 + NH_4^+$$

Ammonia and amines are well-known products of putrefaction, the chief processes being deamination and decarboxylation of amino acids derived from proteins (*5*).

Fish are excellent for testing the hypothesis of precipitation of $CaCO_3$ by organic decay. A common test for spoilage during the storage and processing of fish for use as food is the measurement of *p*H; any slight rise above the characteristic value for fresh fish is often used to indicate putrefaction. With this in mind and the knowledge that fish are generally rich in basic proteins, an experimental investigation of long-term bacterial decay of fish in seawater and other solutions was undertaken to see if a rise in *p*H could be maintained over an extended period and whether actual precipitation of $CaCO_3$ could be induced.

Headless, uncleaned, fresh, whole smelts and butterfish were placed singly in stoppered jars containing liquids approximately three times the volume of the fish in order to provide a high ratio of fish to water and to promote anaerobic conditions within the jars (*6*). The liquids used were filtered seawater from Long Island Sound, 0.125*M* $CaCl_2$ solution, and distilled water. No inoculations of specific bacteria were made. Decomposition was allowed to take place, and from time to time measurements were made of *p*H *in situ*, and of the concentrations of dissolved calcium, magnesium, total carbonate, and ammonia plus volatile amines. Occasional analyses were made for sulfate and chloride to check for bacterial reduction of sulfate and for evaporation or dilution by fish body fluids. No appreciable changes in either sulfate or chloride were found. The following techniques were used in the measurements: for *p*H, an electrometric method using glass plus calomel electrodes; for dissolved calcium and magnesium, titration with ethylenediaminetetraacetic acid at higher concentrations and atomic absorption spectrophotometry at low concentrations; for total dissolved carbonate, acidification, boiling, reaction with $Ba(OH)_2$ solution, and weighing as $BaCO_3$; and for ammonia plus volatile amines, micro-Kjeldahl distillation plus titration of excess acid with NaOH. Sulfate and chloride were measured titrimetrically. All samples of the solutions were passed through a Millipore filter (pore size, 0.45 μ) prior to analysis. A Siemans x-ray diffraction goniometer employing pulse height analysis was used to test for precipitated "inorganic" solids, and a special low-angle gold wedge was used to check for peaks corresponding to very high angstrom spacings. Samples of partially disaggregated fish slurry were taken and were treated with 30 percent H_2O_2 for about 1 week at room temperature to remove as much amorphous organic material as possible. The fine white material remaining in suspension was then collected and mounted on slides for x-ray analysis. This enabled the exclusion of coarse bone material and other hard parts that might show an interfering x-ray pattern.

Decomposition was continued and chemical and x-ray analyses were made over total periods ranging from 65 to 205 days. Results for several representative runs are shown in Fig. 1 and Table 1. In all, seven runs were made with butterfish and four with smelt. Replicate runs of the same solution showed little between-sample variation. The striking results shown are the large increase in *p*H, the precipitation of dis-

Table 1. Effect of decomposition of butterfish on the composition and *p*H of seawater and of a solution of $CaCl_2$.

Ion	Concentration of ion (mmole/liter)			
	Original sea water	Sea water after 135 days	Original solution of $CaCl_2$ (0.125*M*)	Solution of $CaCl_2$ (0.125*M*) after 205 days
Ca^{++}	8.5	0.5	125	5
Mg^{++}	45.5	10.4	0	0
NH_4^+	0.0	220	0	450
$CO_3^=$, HCO_3^-	1.8	120	0	110
	*p*H *values*			
	6.5 (with fish)	8.8	5.8 (with fish)	8.0

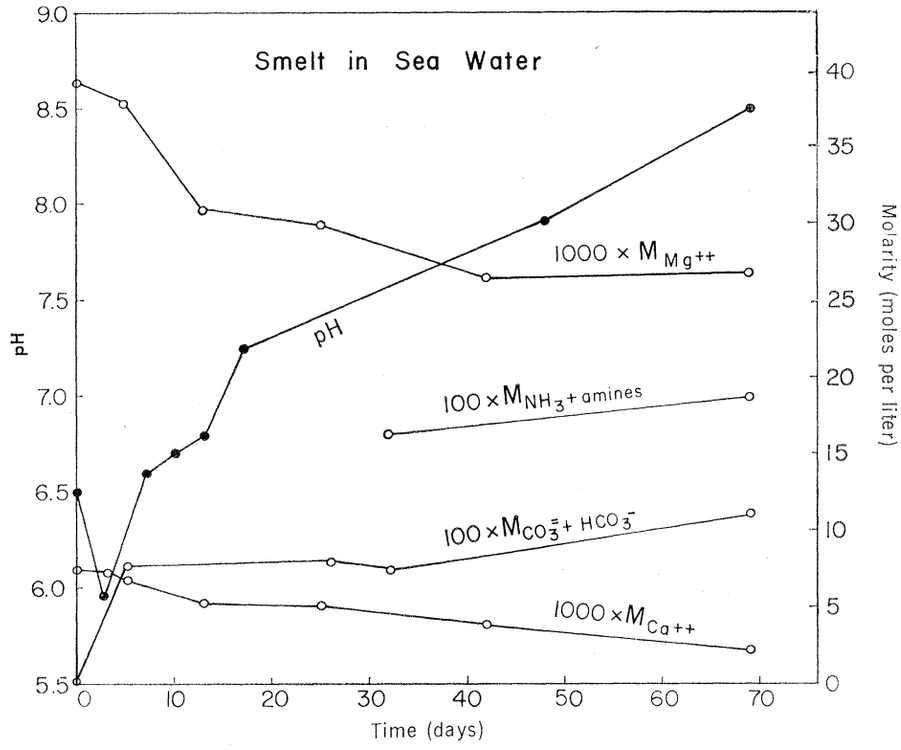

Fig. 1. Chemical changes in seawater during initial bacterial decomposition of a smelt at room temperature.

solved calcium and magnesium, and the very large increases in the concentrations of total dissolved carbonate and ammonia plus volatile amines. These chemical changes drastically alter the chemical composition of seawater, as can be seen from Table 1.

The rise in *p*H is directly due to the release of ammonia and other bases to solution. Besides raising the *p*H, ammonia also reacts with bacterially generated CO_2 to form dissolved carbonate and bicarbonate ions:

$$CO_2 + NH_3 + H_2O \rightarrow NH_4^+ + HCO_3^-$$
$$CO_2 + 2NH_3 + H_2O \rightarrow 2NH_4^+ + CO_3^=$$

Because of the rise in *p*H, the formation of high concentrations of carbonate ion, and the loss of calcium from solution, it was confidently expected that $CaCO_3$ would be found as a precipitate in the jars. This was not the case for any run, which was especially surprising because calculated values of the products of ion activity, for calcium and carbonate ions in the solutions, far exceeded that expected for saturation. In seawater and $CaCl_2$ solution the ion product averaged about 50 times the saturation value. High supersaturation was also demonstrated by the precipitation of ≈85 percent of the calcium in each of two samples after the addition of a large amount of calcite.

Instead of calcite or aragonite, another x-ray diffracting substance was discovered that is characterized by three prominent low-angle maxima. The peaks correspond to a mixture of calcium (and possibly some magnesium) salts of fatty acids or soaps with from 14 to 18 carbon atoms. Positive identification was made by comparison with x-ray patterns for pure calcium or magnesium myristate, palmitate, oleate, and stearate. Results for a representative sample are shown in Fig. 2. The x-ray patterns indicate a preponderance of calcium over magnesium although more magnesium than calcium was precipitated in the seawater runs. This was corroborated by chemical analyses for Ca and Mg (*7*). Apparently, most magnesium was incorporated into nondiffracting compounds or reacted with coarse bone material.

The finding of calcium salts of fatty acids with 14 to 18 carbon atoms as a peroxide-resistant residue was not surprising because of the large amounts of fatty acids in this carbon range in the fat of fishes (*8*). Upon fat hydrolysis by bacteria the free fatty acids can react with ammonia to form soluble ammonium soaps which in turn precipitate as insoluble calcium soaps. The soap-forming reactions are well known and can be readily demonstrated artificially. Thus, the production of ammonia and other bases promote the formation of calcium soaps as well as of $CaCO_3$.

The absence of $CaCO_3$ and presence of calcium salts of fatty acids can be explained by thermodynamic stability. Based on the solubility products of free fatty acids and their calcium salts in water (*9*) and thermodynamic data for $CaCO_3$, CO_2, and H_2O (*10*), the equilibrium constant K at 25°C for the reaction

$$2\ RCOOH + CaCO_3 \leftrightharpoons Ca(RCOO)_2 + CO_2 + H_2O$$

can be calculated to be $K = P_{CO_2} = 500$ atm for $R=C_{13}H_{27}$ (myristate), 7×10^4 atm for $R=C_{15}H_{31}$ (palmitate), and 3×10^6 atm for $C_{17}H_{35}$ (stearate). Therefore, at least for saturated acids, $CaCO_3$ is thermodynamically unstable in the presence of excess free fatty acid at the much lower partial pressures of carbon dioxide encountered in natural waters and sediments. Calcium carbonate can accumulate stably only after all excess free fatty acid is bound to calcium. Although solubility data are scanty and in relatively poor agreement for ammonium soaps, it appears that $CaCO_3$ may be unstable in the presence of saturated solutions of ammoniated as well as of free acids.

Formation of natural calcium salts of fatty acids, called adipocere, by the decay of dead organisms, including humans as well as fish, has been noted by many investigators (*11*). Adipocere

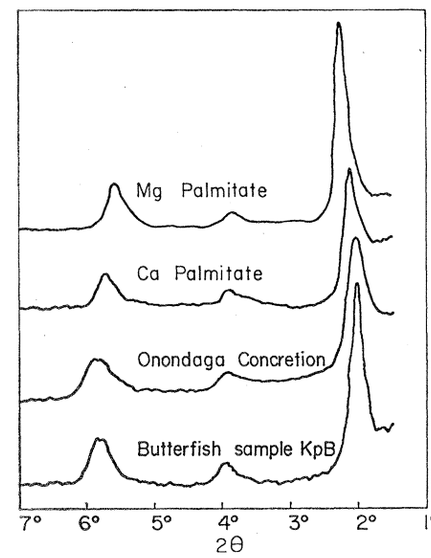

Fig. 2. X-ray diffraction patterns for pure Ca and Mg palmitate, adipocere formed from decomposition of butterfish in the laboratory, and an alewife concretion from Lake Onondaga (Cu$K\alpha$ radiation).

often replaces and takes the form of the decomposing body and forms concretions retaining much of the external morphology of the organism. A recent example is the occurrence of alewife concretions described by Sondheimer *et al.* (*12*) from calcium-rich Lake Onondaga. The x-ray diffraction pattern of one of these concretions is shown in Fig. 2 and is very similar to that of the material formed in the laboratory. The chemical composition of the Onondaga concretion (which consists mainly of calcium palmitate) is almost identical (in C:H:Ca+Mg ratio) to herring adipoceres described by Wells and Erickson (*13*) which had formed in seawater off the coast of Alaska.

Various mechanisms for the breakdown of fatty acids as processes involved in the formation of petroleum have been suggested by many workers (*14*). If such breakdown actually occurs, then an original adipocere concretion may eventually become a $CaCO_3$ concretion once the hydrocarbon portion is removed. Excellent preservation of fossils within $CaCO_3$ concretions is believed to be due to very rapid syngenetic formation of the concretion on or near the sea floor (*1*). If some concretions originally consisted of adipocere, such a rapid fossilization mechanism is reasonable in the light of the present laboratory experiments and the finding of adipoceres only tens of years old (*11*). The present experiments also demonstrate that even in the absence of fatty acid, abundant alkalinity is available from the decomposition of fish to precipitate calcium as $CaCO_3$ during the early stages of decay.

The conversion of adipocere to $CaCO_3$ because of a considerable density increase, would necessitate a shrinkage of the concretion. Although this effect should not greatly affect an enclosed skeleton, it could destroy external morphology. The finding of $CaCO_3$ replacements preserving external features, as described by Weeks (*1*), would necessitate a virtually isotropic shrinkage or later infilling by $CaCO_3$ from ground water, if the concretions were derived from original adipocere.

One possible test of an original adipocere origin for $CaCO_3$ concretions is the measurement of the C^{13}/C^{12} ratio of the carbonate. Adipocere and lipid carbon are highly depleted in C^{13} relative to normal marine calcium carbonate (*12*). During the breakdown of adipocere it is probable that the remaining calcium will form $CaCO_3$ from isotopically light carbon. Thus $CaCO_3$ concretions containing well-preserved fossils, which show a very low C^{13}/C^{12} ratio in the carbonate, may have formed originally as adipocere which was later converted to $CaCO_3$. Nonfossiliferous concretions very low in C^{13}, such as some of those described by Hodgson (*15*), may have formed in a similar manner.

ROBERT A. BERNER
Department of Geology, Yale University, New Haven, Connecticut

References and Notes

1. W. H. Twenhofel, *Treatise on Sedimentation* (Dover, New York, 1965), p. 714; L. G. Weeks, *Bull. Geol. Soc. Amer.* **68**, 95 (1957).
2. A good example is shown by the table in K. O. Emery, *The Sea off Southern California* (Wiley, New York, 1960), p. 276.
3. H. R. Krause, *Arch. Hydrobiol.* **24**, 297 (1959).
4. F. E. Hecht, *Senckenberg. Naturforsch. Gesellschaft* **15**, 165 (1933).
5. K. V. Thimann, *The Life of Bacteria* (Macmillan, New York, 1963).
6. No special precautions against aeration were taken during withdrawal of samples for chemical analysis or during measurement of *p*H. The presence of considerable dissolved sulfide (from breakdown of sulfur-containing organic compounds) as measured with a Ag_2S electrode was found in six out of seven runs after 120 to 170 days of rotting. This indicates the ability to reattain anaerobic conditions even after repeated opening of the jars and exposure to air. Also, aeration and decrease in volume due to sample withdrawal brought about no qualitative difference in chemical changes with time between runs sampled very infrequently ($<$ 10 percent volume decrease) and those sampled frequently ($>$ 50 percent volume decrease).
7. Chemical analysis of part of the material remaining in suspension after treatment with H_2O_2, which could be centrifuged down after 1 hour at 2000 rev/min, showed Ca/Mg $\sim$ 4 for one sample that had an x-ray pattern matching Ca fatty acid salts. Another sample showing an x-ray pattern for Ca salts had Ca/Mg $\sim$ 6. This sample was collected by settling in water for about 1 month.
8. E. H. Gruger, R. W. Nelson, M. E. Stansby, *J. Amer. Oil Chem. Soc.* **41**, 662 (1964).
9. The solubility products for the free fatty acids are based on the data of L. M. John and J. W. McBain, *J. Amer. Oil Chem. Soc.* **40** (1948). These values are lower than those of A. W. Ralston and C. W. Hoerr [*J. Org. Chem.* **7**, 546 (1942)] so that equilibrium values of P_{CO_2} may be even higher. Solubility product constants for calcium soap are taken from the precise work of R. R. Irani and C. F. Callis [*J. Phys. Chem.* **64**, 1741 (1960)].
10. R. M. Garrels and C. L. Christ, *Solutions, Minerals, and Equilibria* (Harper and Row, New York, 1965).
11. For an excellent discussion and summary of references on adipocere see W. Bergmann, in *Organic Geochemistry*, I. A. Breger, Ed. (Pergamon, New York, 1963), p. 517.
12. E. Sondheimer, W. A. Dence, L. R. Mattick, S. R. Silverman, *Science* **152**, 221 (1966).
13. R. C. Wells and E. T. Erickson, *J. Amer. Chem. Soc.* **55**, 338 (1933).
14. I. A. Breger, *Geochim. Cosmochim. Acta* **19**, 297 (1960); J. E. Cooper and E. E. Bray, *ibid.* **27**, 1113 (1963); W. D. Rosenfeld, *Arch. Biochem.* **16**, 263 (1948); S. R. Silverman, in *Isotopic and Cosmic Chemistry*, H. Craig, S. L. Miller, G. J. Wasserburg, Eds. (North-Holland, Amsterdam, 1964), p. 92.
15. W. A. Hodgson, *Geochim. Cosmochim. Acta* **30**, 1223 (1966).
16. Supported by grant 1937-A2 of the Petroleum Research Fund, American Chemical Society. Technical assistance was provided by Mrs. C. Thomlinson; concretions from Lake Onondaga were provided by E. Sondheimer.

14 August 1967

Dense-Gas Chromatography of Nonvolatile Substances of High Molecular Weight

Abstract. *Working at pressures of up to 2000 atmospheres, more than ten times higher than in previous gas chromatography, we used the solvent power of dense gases to enable migration of chromatographic substances of molecular weights as high as 400,000. Carotenoids, corticol steroids, sterols, nucleosides, amino acids, carbohydrates, and several polymers have been caused to migrate, separated, and detected in NH_3 and CO_2 carrier gases at temperatures of 140° and 40°C, just above the respective critical points. Previously such compounds either defied separation by gas chromatography or had to be chromatographed as their more volatile derivatives.*

Gas chromatography has been spectacularly successful for high-resolution, high-speed separation. Its range has been strictly limited, however, by the requirement that all substances must be reasonably volatile in order to achieve substantial partitioning with the gas phase; thus excluded are many challenging separations involving biochemical, inorganic, and macromolecular substances (*1*). This limitation can be moderated or even eliminated by use of dense-gas chromatography.

Dense gases promise to constitute an especially versatile class of mobile phases for chromatography; depending on the degree of compression and on the temperature relative to the critical point, their properties can range continuously between the extremes of ordinary liquids and ideal gases. Since liquids are approximately 1000 times denser than gases at atmospheric pressure, compression of gases to roughly 1000 atm yields fluids whose properties are generally liquid-like. Intermolecular forces play a dominant role and give the fluid a solvent power resembling that of a true liquid. If the proper gas is chosen, considerable solvent power may remain even when density (and pressure) is lowered from the level at which the gas closely simulates liquids. Thus one has considerable latitude in choosing pressure and temperature at which the gas will exhibit favorable solvent effects while still largely retaining the favorable viscous and diffusive characteristics that are fundamentally responsible for the advantages of gas chromatography. Moreover, one may