data indicate that the stable isotope composition of sulfur pools in *Spartina alterniflora* demonstrates the uptake and internal oxidation of sulfide by the plant and may be useful in determining sulfide uptake by other plant species growing in anaerobic sediments.

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8. $$\delta^{34}S = \frac{^{34}S/^{32}S_{sample} - {}^{34}S/^{32}S_{standard}}{^{34}S/^{32}S_{standard}} \times 100$$

where $^{34}S/^{32}S_{standard}$ refers to Canyon Diablo meteoritic hydrotroilite and $\delta^{34}S$ has the units per mil (*7*).
9. Our experimental methods may be summarized as follows. The plants (all representing the medium-height form of *Spartina*) were washed free of sediment and transferred to aerated hydroponic culture from marsh sediments in March 1979. We gradually salinized the hydroponic medium by replacing transpired water with Instant Ocean water until a salinity of 35 per mil was attained. The medium was aerated constantly and replaced weekly until 5 September 1979, when the plants were harvested. During the 6-month period, the culms increased in wet weight from between 0.8 and 1.0 g to between 7 and 10 g. Aboveground tissue was separated from roots and rhizomes, washed in distilled water, dried to constant weight at 60°C, and ground in a Wiley mill. On 23 August 1979, aboveground tissue of *Spartina* was collected from plants in the same marsh from which the hydroponically grown plants had been collected. Cores were also taken from the marsh sediment for the determination of concentrations and isotopic composition of sulfate and sulfide in pore water.

   Our analytical procedure consisted of the following. Pore water was separated from 2-cm-thick segments of cores by centrifugation. Sulfide (5-ml aliquots) was precipitated with zinc acetate and subsequently acidified and swept by a nitrogen stream into aqueous silver chloride and precipitated as silver sulfide. Pore-water sulfate and sulfate within the plants were determined gravimetrically after precipitation as barium sulfate. Total plant sulfur was also determined gravimetrically as sulfate after combustion in a Parr oxygen bomb. For isotopic analyses, barium sulfate precipitates and total sulfur analyses were reduced with a mixture of hydriodic, phosphorous, and hydrochloric acids to form hydrogen sulfide, which was swept in a nitrogen stream and collected as silver sulfide. The isotopic composition of the sulfide was determined by combustion of the silver sulfide in a stream of oxygen and mass spectrometry of the resultant sulfur dioxide by the procedure of J. Forrest and L. Newman [*Atmos. Environ.* **7**, 561 (1973)].
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Curiosities in Periodic Precipitation Patterns

Abstract. *Observations have been made of several types of Liesegang patterns that are more complex than the usual concentric rings or parallel bands of precipitate. The structures observed show radial gaps, segmentation, irregular patterns within concentric rings, and the formation of spiral precipitation bands instead of sets of parallel Liesegang bands.*

In a series of investigations of periodic precipitation processes, in part reported in (*1*, *2*), we found some interesting curiosities to be described briefly here. In a standard Liesegang experiment a soluble electrolyte diffuses into a solution, containing another electrolyte, to which a gel-forming material has been added. On interdiffusion a chemical reaction occurs in which a weakly soluble salt is formed and precipitated in discontinuous bands parallel to the front of the diffusing electrolytes (*3*). In an effectively "one-dimensional" Liesegang experiment the two electrolyte solutions are placed in a cylindrical container, such as a test tube, and a set of parallel bands of precipitate appears perpendicular to the axis of the test tube. A typical "two-dimensional" Liesegang system is prepared in a flat container, such as a petri dish, so that initially a thin circular section of one of the two solutions is surrounded by a layer of the second electrolyte medium. Interdiffusion takes place in the radial direction and the resulting pattern consists of concentric rings of precipitate. Depending on the initial salt concentrations, the rings form either in the inner or in the outer section of the container. In this report we present observations of radial gaps, pocket-like structures, segmentation, and irregular patterns within concentric Liesegang rings and of spiral precipitation bands in one-dimensional Liesegang experiments (*4*).

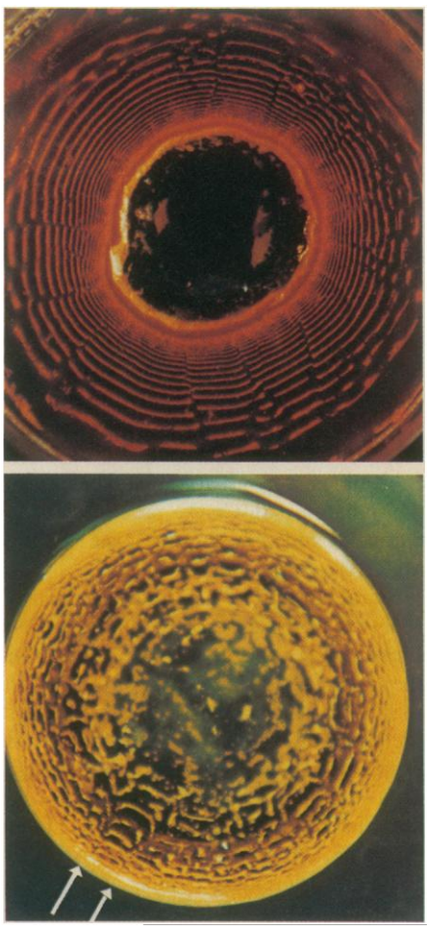

Fig. 1. Radially aligned gaps in concentric rings of PbI_2 precipitate in Liesegang systems with ring formations away from (top) and toward the center of (bottom) a petri dish, after 5 days. The arrows point to one of the regions in which a radial set of pocket-like structures appears. Initial electrolyte concentrations in the inner and outer sections of the containers: (top) 0.12*M* KI and 0.012*M* $Pb(NO_3)_2$; (bottom) 0.006*M* $Pb(NO_3)_2$ and 0.12*M* KI with 0.3*M* $Ba(NO_3)_2$ added (*16*). All solutions contained 1 percent agar-agar gel. Nearly to scale.

We obtained two-dimensional Liesegang structures with lead iodide as the precipitate by covering the bottom of a petri dish (diameter, 90 mm) with a layer 1 to 4 mm thick of 0.005 to 0.015*M* $Pb(NO_3)_2$ in 0.5 to 1.0 percent agar gel. After the gel had solidified we removed a circular portion of the layer (diameter, 30 to 60 mm) from the center of the dish and subsequently filled this empty section to the same height with 0.06 to 0.12*M* KI also containing 0.5 to 1 percent agar (*5*). Within a period of typically 2 days a set of concentric rings of PbI_2 precipitate appeared in the lead solution [outer region of the dish (Fig. 1, top)]. Ring formation occurred in the inner circular section when the $Pb(NO_3)_2$ solution was placed in the center of the dish and the

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KI solution occupied the surrounding area (Fig. 1, bottom) (*6*). In many but not all experiments we found that these rings consist of a number of convex segments of PbI_2 that are separated from each other by small gaps in which no precipitate can be detected visually (*7*). A comparison of the locations of these gaps in subsequent rings shows that they extend out across the ring system in roughly radial directions. This is illustrated by the example in Fig. 1, top, for the case of ring formation in the outer part of the gel layer; the pattern has a similarity to the spokelike features in Saturn's rings which revolve around the planet (*8*). In Fig. 1, bottom, we show the occurrence of rhythmic precipitation in the inner circular section of the container. There is some concentric ring structure, but more complex pattern formations are superposed. Note, for instance, the pocket-like structures and their radial alignment in the segment between the arrows (*9*).

Examples of the formation of additional types of patterns within concentric Liesegang rings of PbI_2 precipitate are given in Fig. 2. We frequently observed that some portions of the rings are divided into many small, approximately parallel segments or stripes on the order of 0.5 mm long, as shown in Fig. 2A. Figure 2B is an oblique photograph of a section of another two-dimensional ring system (actually a set of concentric cylinders of the height of the gel layer). This view reveals that within each ring regions of high and low translucence alternate in an irregular manner; these correspond to regions of low- and high-density precipitate of PbI_2. This internal ring structure is somewhat reminiscent of spatially inhomogeneous precipitation patterns that develop from initially homogeneous supercooled PbI_2 solutions (gradient-free structures) (*10, 11*).

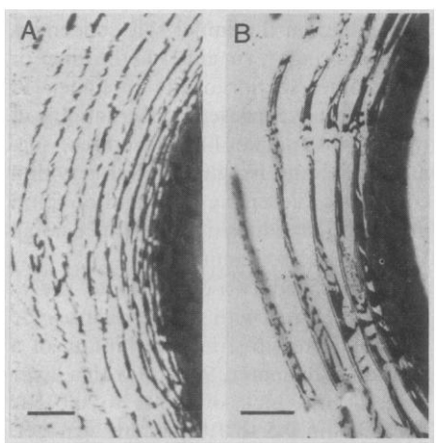

Fig. 2. Patterns within concentric Liesegang rings of PbI_2. (A) Several ring sections partially consisting of small, approximately parallel segments. Initial electrolyte concentrations: 0.12*M* KI (inner section of dish) and 0.012*M* $Pb(NO_3)_2$ (outer section of dish). Both solutions contained 1 percent agar-agar gel. (B) Ring system viewed from an oblique angle with irregular patterns within each ring. Initial electrolyte concentrations: 0.06*M* $Pb(NO_3)_2$ (inner section) and 0.015*M* KI (outer section) (*6*). Both solutions contained 0.7 percent agar-agar gel. Patterns (A) and (B) are shown 5 days after the start of the experiment. Scale bars: (A) 2 mm and (B) 5 mm.

We investigated the formation of one-dimensional, banded Liesegang patterns in test tubes 5 mm in diameter for the following salts: $MgSO_4$ in gelatin and NH_4OH forming $Mg(OH)_2$ bands in the $MgSO_4$ solution; KI in agar and $Pb(NO_3)_2$ in agar forming PbI_2 bands in either of the two solutions; and $K_2Cr_2O_7$ in gelatin and $AgNO_3$ forming $Ag_2Cr_2O_7$ bands in the $K_2Cr_2O_7$ solution (*5*). For all these systems we observed in several cases the development of a spiral (or helicoidal) precipitation band instead of parallel and well-separated bands of precipitate. Spirals of $Mg(OH)_2$ and $Ag_2Cr_2O_7$ are shown in Fig. 3, A and B. They extend from the initial junction between the two electrolyte solutions down the tube. The $Mg(OH)_2$ spiral occupies a region in which the first three rings are usually located. Farther down the tube the common banded structures appear, as seen in Fig. 3A, where the final precipitation pattern is shown. The picture of the $Ag_2Cr_2O_7$ tube in Fig. 3B was taken at a relatively early stage of the pattern evolution (after 1 day); at later times and greater distances from the junction $Ag_2Cr_2O_7$ precipitated in parallel bands, as in the case of the $Mg(OH)_2$ spiral.

Spiral formation has been frequently reported in the case of two-dimensional Liesegang experiments, especially for the precipitation of $Ag_2Cr_2O_7$ (*12*). In regard to one-dimensional experiments the observation of spiral formation has been mentioned, but to our knowledge little evidence has been presented in the literature (*13*). We obtained spirals under the same experimental conditions (*2*) as those for Liesegang bands, and we do not know what differences lead to one type of structure or the other. Care was taken not to introduce any changes in the experimental procedure; thus at present the occurrence of spirals must be considered a random event of low probability. Spirals tend to form more frequently when high rather than low electrolyte concentrations are used, that is, when the concentration gradients close to the junction are large. This finding can be related to the fact that with increasing distance from the junction (and decreasing concentration gradients) the helicoidal bands are replaced by parallel bands. Among the few observed examples we saw both right- and left-handed spirals with about equal probability.

There is experimental evidence that structure formation in precipitating systems with or without initially imposed gradients of electrolyte concentration is a postnucleation phenomenon (*1, 11*). This lends support to theories in which the structure formation is associated with a chemical instability due to the competitive growth of colloidal particles (*11, 14*). Investigations of Liesegang and gradient-free systems by ordinary light microscopy point to a colloidal composition of the precipitated salt, which indicates that colloidal rather than epitaxial growth leads to the final visible patterns (*15*). Furthermore, recent studies of the Liesegang phenomenon have shown that the precipitation patterns have reproducible locations in electrolyte systems in which strong initial concentration gradients are imposed; when these gradients are lowered, the structures become increasingly random in spatial location (*2*). A stochastic element prevails in the case of inhomogeneous pattern formation in gradient-free precipitating sys-

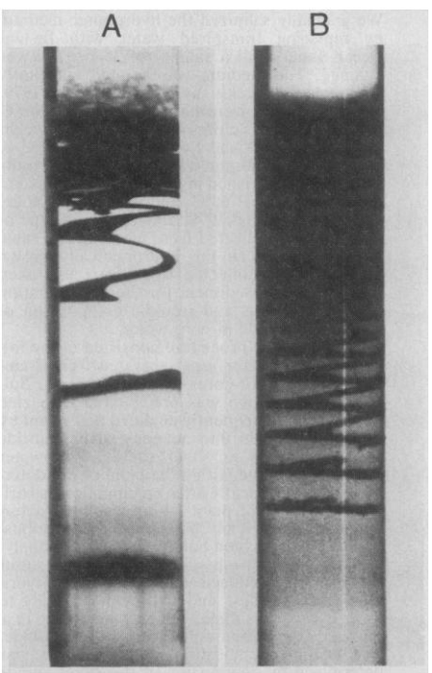

Fig. 3. (A) Spiral band of $Mg(OH)_2$ after 4 days. Initial electrolyte solutions: 5.5*M* NH_4OH (upper portion of tube) and 0.37*M* $MgSO_4$ in 8 percent gelatin (lower portion of tube). (B) Spiral band of $Ag_2Cr_2O_7$ after 1 day. Initial electrolyte solutions: 0.92*M* $AgNO_3$ (upper portion) and 6.8 m*M* $K_2Cr_2O_7$ in 5 percent gelatin (lower portion). Inner diameter of tubes, 5 mm.

tems (*2, 10, 11*). The curiosities reported here provide further evidence of the complexity of patterned precipitation.

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4. The term "secondary Liesegang structures" frequently appears in the literature; it usually refers to the separation of Liesegang bands into several thinner bands, mostly observed for $Ag_2Cr_2O_7$ [K. S. Ramaiah, *Proc. Indian Acad. Sci. Sect. A* **9**, 467 (1939)].
5. The preparation of Liesegang experiments is described in detail in (*1, 2*).
6. Rings of PbI_2 are also formed in the iodide solution, provided that the lead concentration sufficiently exceeds the iodide concentration (compare Fig. 2B).
7. We confirmed that the gaps are not due to fractures in the gel.
8. For example, see *Sci. News* **120**, 107 (1981).
9. A different type of radial structure is a set of radially aligned dislocations, observed for Ag_2CrO_4 rings [C. V. Raman and K. S. Ramaiah, *Proc. Indian Acad. Sci. Sect. A* **9**, 455 (1939)].
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16. The presence of $Ba(NO_3)_2$ affects the details of the patterns but is not necessary for the appearance of radial features.
17. Supported in part by the National Science Foundation and by the Air Force Office of Scientific Research. A fellowship from the Deutsche Forschungsgemeinschaft for S.C.M. is gratefully acknowledged.
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Human Aldehyde Dehydrogenase: Mechanism of Inhibition by Disulfiram

Abstract. *Disulfiram labeled with carbon-14 reacts specifically with human liver aldehyde dehydrogenase E_1 with loss of catalytic activity and no incorporation of label. Carbon-14–labeled diethyldithiocarbamate is formed and the number of enzyme sulfhydryl groups decreases from 34 to 30 during this process. Activity is recovered by 2-mercaptoethanol but not by glutathione, the physiological reducing agent.*

Disulfiram (tetraethylthiuram disulfide) is used therapeutically in the treatment of alcoholism. Its administration before drinking of alcoholic beverages results in unpleasant symptoms such as blurred vision, nausea, and flushing of the face and neck. Disulfiram apparently acts by inhibiting aldehyde dehydrogenase (E.C. 1.2.1.3) (*1*), causing elevation of blood acetaldehyde after consumption of ethanol (*2*). The mechanism by which disulfiram inhibits hepatic aldehyde dehydrogenase has been investigated for the past 20 years. In 1966 disulfiram was shown by Neims *et al.* (*3*) to be a sensitive, general reagent for the modification of protein sulfhydryl groups by the reaction shown in Fig. 1A.

Studies with purified cytoplasmic and mitochondrial aldehyde dehydrogenases from various species (*4–7*) indicate that cytoplasmic enzyme is more susceptible to disulfiram inhibition than mitochondrial enzyme. Activity lost after treatment with disulfiram is recovered with 2-mercaptoethanol or 1,4-dithiothreitol but not after prolonged dialysis, suggesting formation of a covalent bond between disulfiram and enzyme by a mechanism similar to that described in Fig. 1A. There are, however, numerous enzymes with essential sulfhydryl groups that are not inhibited by disulfiram; in addition, Strömme (*8*) has reported that soluble rat liver proteins incorporate little radioactivity after in vivo treatment with [^{35}S]disulfiram. The mechanism by which disulfiram specifically inhibits aldehyde dehydrogenase activity is therefore poorly understood.

We report on the mechanism by which human liver cytoplasmic aldehyde dehydrogenase is inhibited by disulfiram. Our results indicate that disulfiram binds at a specific site and oxidizes essential enzyme sulfhydryl groups to form internal S–S bonds.

The E_1 isoenzyme of human aldehyde dehydrogenase, purified to homogeneity by a modification of the procedure of Greenfield and Pietruszko (*9*), was dialyzed against ten changes of N_2-saturated 30 m*M* phosphate buffer (*p*H 7.0) containing 1 m*M* EDTA at 4°C. Also used were glutathione, 2-mercaptoethanol, and 5,5′-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent) (Sigma); disulfiram (Ayerst); ^{14}C-labeled disulfiram (tetraethylthiuram disulfide) (Amersham); sodium arsenite (Baker); and nicotinamide adenine dinucleotide (NAD^+) (Boehringer-Mannheim).

Aldehyde dehydrogenase activity was determined spectrophotometrically at 25°C and 340 nm in 100 m*M* sodium pyrophosphate buffer (*p*H 9.0) containing 1 m*M* EDTA, 900 μ*M* NAD^+, and 260 μ*M* propionaldehyde. Protein concentrations were determined by the method of Lowry *et al.* (*10*) with bovine serum albumin (Sigma) used as a standard. Enzyme was inactivated by mixing with buffer containing disulfiram. Incubations (in 30 m*M* sodium phosphate, *p*H 7.0, containing 1 m*M* EDTA) were kept under nitrogen at room temperature for the desired length of time; portions were drawn to determine enzyme activity and for counting of radioactivity on an Intertechnique Liquid Scintillation Counter. Control and disulfiram-treated enzyme

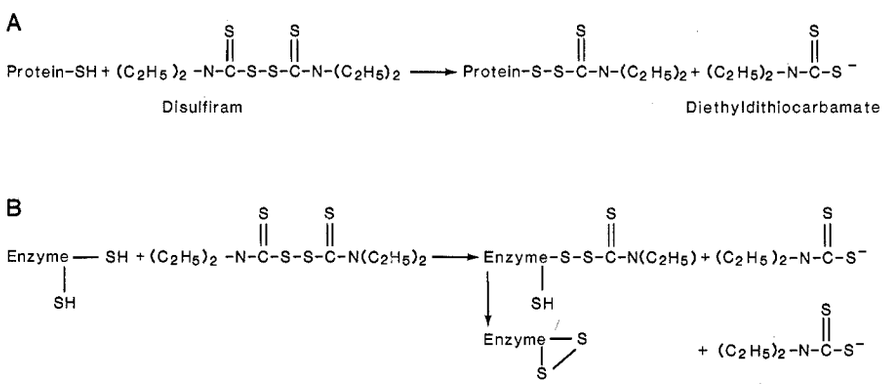

Fig. 1. (A) Reaction proposed by Neims *et al.* (*3*) as the general mechanism by which protein sulfhydryl groups are covalently modified by disulfiram. (B) Proposed mechanism of inhibition of aldehyde dehydrogenase by disulfiram.

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