

ing agents in fire fighting has been repeatedly demonstrated through the years and is beyond question. However, the exact nature of the chemical effect on the degradation of the solid (cellulose or sucrose) or on the reactions in the flame can remain the source of profitable speculation for some time to come.

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24 January 1961

Isotopic Variations in Meteoric Waters

Abstract. The relationship between deuterium and oxygen-18 concentrations in natural meteoric waters from many parts of the world has been determined with a mass spectrometer. The isotopic enrichments, relative to ocean water, display a linear correlation over the entire range for waters which have not undergone excessive evaporation.

Epstein and Mayeda (1) and Friedman (2) reported precise data for O^{18}/O^{16} and D/H ratios in nine non-marine meteoric waters and found a rough linear correlation between the isotopic enrichments. In the course of research on isotopic variations in volcanic waters, I have analyzed mass spectrometrically some 400 samples of water from rivers, lakes, and precipitation in order to establish the exact nature of the isotopic relationship in meteoric waters. Gas samples were prepared by the standard CO_2-H_2O equilibration technique (1) and by reduction of H_2O to H_2 with uranium metal and analyzed on the McKinney-Nier type spectrometers used by the authors mentioned above as well as in my present laboratory.

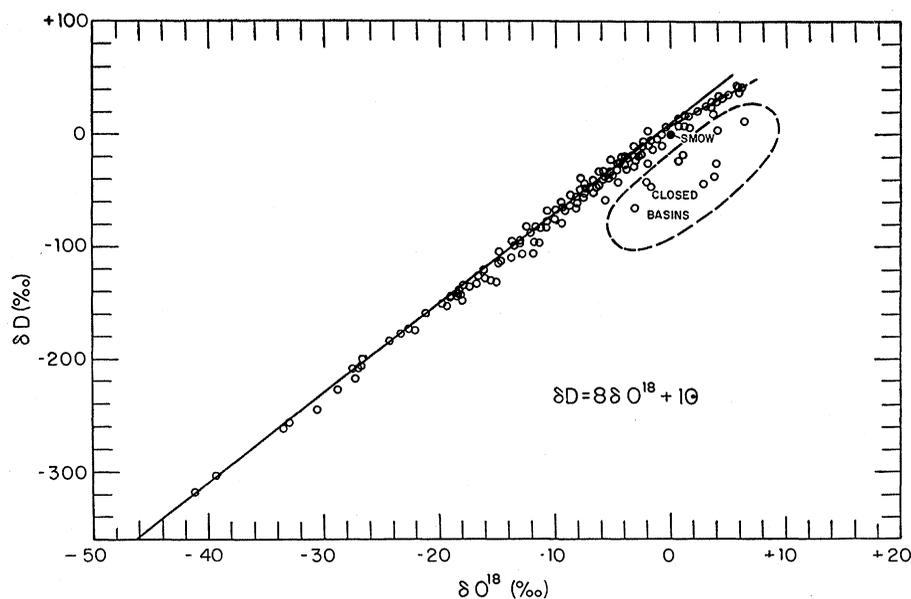


Fig. 1. Deuterium and oxygen-18 variations in rivers, lakes, rain, and snow, expressed as per millage enrichments relative to "standard mean ocean water" (SMOW). Points which fit the dashed line at upper end of the curve are rivers and lakes from East Africa.

The isotopic data for all samples analyzed for both isotopes (excluding detailed sets of data from Chicago and Steamboat Springs, Nev.) are shown in Fig. 1. About 40 percent of the samples are from North America, the rest being distributed all over the world. The data shown are per mil enrichments of the isotopic ratios D/H and O^{18}/O^{16} relative to a mean ocean water standard, that is,

$$\delta = [(R/R') - 1] 1000$$

where R is either isotopic ratio and R' is the ratio in "standard mean ocean water" (SMOW) defined relative to the National Bureau of Standards isotopic water standard as described in a following report (3). The precision of the data is ± 0.5 per mil, or ± 1 percent of δ , for D, and ± 0.1 per mil, or ± 0.5 percent of δ , for O^{18} , the larger error applying in each case and representing ± 2 standard deviations.

The straight line in Fig. 1 represents the relationship

$$\delta D = 8 \delta O^{18} + 10$$

(both δ values in per millage) and is seen to be an adequate fit to the data, except for waters from closed basins in which evaporation is a dominant factor governing the isotopic relationship. The samples which fit the dashed line at the high enrichment end of the curve represent rivers and lakes in East Africa. They fit a line with a slope of about 5, in contrast to the slope of 8 found for most of the data. Studies of evaporation in the laboratory, and in areas where seasonal data have been obtained, show that in free evaporation at ordinary temperatures the heavy iso-

tope enrichment ratio $\delta D/\delta O^{18}$ consistently follows a slope of about 5 as observed in East African waters. Many of the points falling to the right of the line plotted in Fig. 1 have a similar slope of 5 when connected to points on the line which represent direct precipitation in the same area.

It can be shown (4) that for small enrichments the slopes in Fig. 1 are the ratios of the single-stage enrichments when the isotopic concentrations are governed by vaporization or precipitation under Rayleigh conditions at constant temperature. The isotopic vapor pressure data show that slopes of 8 and 5 correspond to Rayleigh processes at liquid-vapor equilibrium at temperatures of about $-10^\circ C$ and $+100^\circ C$ respectively. It seems, therefore, that atmospheric precipitation follows a Rayleigh process at liquid-vapor equilibrium, as first proposed by Kirshenbaum (5), but that the process of free evaporation at room temperature is governed by kinetic factors. The present studies have shown that this is so up to the boiling point, and that the disequilibrium occurs principally in the O^{18}/O^{16} separation (4). Some of the variability along the line in Fig. 1 is certainly due to evaporation effects as well as to variations in temperature of precipitation.

All points in Fig. 1 for δD and δO^{18} lighter than -160 and -22 per mil, respectively, represent snow and ice from the Arctic and Antarctic, while tropical samples show very small depletions relative to ocean water. This distribution is expected for an atmospheric Rayleigh process as vapor is removed from poleward moving tropospheric air. However, it is actually $\log(1 + \delta)$

which should be plotted for such a process, and, in such a plot, the points in Fig. 1 fall on a curve with a continually increasing slope for lighter δ values, as would be expected (from the vapor pressure data) for precipitation at lower temperatures in high latitudes. The linear relation observed in Fig. 1 simply reflects a coincidence of the effect of the increasing difference in δ and $\log(1 + \delta)$ at high enrichments with the effect on the slope of the average temperature decrease for precipitation along a meridian from equator to poles (6).

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6. Detailed papers on isotopic variations in meteoric and volcanic waters of specific areas will be published elsewhere. It is a pleasure to acknowledge my gratitude to Harold C. Urey in whose laboratories at the Institute for Nuclear Studies, University of Chicago, most of this work was done, to Mrs. T. Mayeda for her excellent services in the Chicago laboratory, and to G. Boato for many interesting discussions. This research has been supported by the National Science Foundation, the University of California Water Resources Commission, the Office of Naval Research, and the Atomic Energy Commission.

18 January 1961

Germination of Bacterial Endospores with Calcium and Dipicolinic Acid

Abstract. Aerobic and anaerobic bacterial endospores can be germinated if calcium chloride and dipicolinic acid are added to well-washed suspensions. Maximum germination is obtained when the calcium and acid are present in a molar ratio of one or more. This suggests that the 1:1 chelate of calcium and dipicolinic acid is the agent that induces germination.

During experiments concerned with the effect of chelating agents on spore germination, we observed that germination was induced when calcium chloride and dipicolinic acid (2,6-pyridine dicarboxylic acid) were added to suspensions of clean, well-washed bacterial spores provided that the molar ratio of calcium to dipicolinic acid was 1:1 or higher.

The procedure was to dissolve the dipicolinic acid in enough NaOH solution to give a neutral solution of known strength. A standard CaCl₂ solution, either with or without tris buffer, was

mixed with the solution of dipicolinic acid and NaOH immediately before addition to the spore suspension. In such mixtures of calcium and dipicolinic acid, we have been able to germinate spores of the following organisms: Putrefactive anaerobe 3679 (NCA and h strains), putrefactive anaerobe S₂, *Clostridium perfringens*, *Bacillus cereus*, *B. megaterium*, *B. mycoides*, *B. subtilis*, and *B. coagulans*. The germination was sometimes incomplete, but in most cases rapid and complete germination took place.

The effect of calcium and dipicolinic acid on germination seemed to be rather specific. None of the following metal ions could be substituted for calcium: Na⁺, K⁺, Mg²⁺, Mn²⁺, Ba²⁺, Co²⁺, Zn²⁺, Cu²⁺, Ni²⁺, Fe³⁺. Neither could other chelating agents or any of the other pyridine dicarboxylic acids be substituted for dipicolinic acid.

Table 1 shows that germination with calcium and dipicolinic acid, as measured either by counting residual (heat-resistant) spores or by counting the dark and the refractile spores with a phase-contrast microscope, is quite rapid.

The effect of different ratios of calcium and dipicolinic acid was tested by adding various concentrations of the acid to a germination solution containing 40 mmole of CaCl₂. Figure 1 shows the results obtained with *Bacillus megaterium* and putrefactive anaerobe S₂.

Chelation takes place when dipicolinic acid is added to a solution containing calcium ions. The calculated concentrations of the 1:1 chelate of calcium and dipicolinic acid are also plotted on the graph. The relationship between the two curves provides a strong suggestion that this chelate is the active inducer of germination. The calculation of the concentration of the 1:1 chelate of calcium and dipicolinic acid was based on data obtained by titrations of the acid with NaOH in the presence and absence of calcium ions. These titrations showed that two types of chelates with different stability constants were formed. If the molar concentration of calcium equals or exceeds that of dipicolinic acid, a chelate is formed between 1 mole of calcium ion and 1 mole of the acid. If the concentration of dipicolinic acid is increased over that of calcium, the formation of a higher chelate containing 2 moles of the acid per mole of calcium ion takes place. That this 1:2 chelate apparently has little or no germination-stimulating effect is also indicated in Fig. 1. This has been further substantiated by similar experiments with other organisms showing that germination in the presence of optimum calcium (40 mmole) is decreased by excess dipicolinic acid. The addition of excess calcium to a

Table 1. Germination of *Bacillus megaterium* and putrefactive anaerobe S₂ spores in 40 mmole of CaCl₂, 40 mmole of dipicolinic acid, and 10 mmole of tris buffer at pH 7.0.

Incubation (min)	Germination (%) based on	
	Phase microscopy	Pasteurized counts
	<i>B. megaterium</i> *	
0	0	0
5	0	
10	81	100
20	99	100
	<i>Putrefactive anaerobes S₂</i> †	
0	0	0
5	93	98
10	99	100

* Incubation temperature, 25°C. † Incubation temperature, 35°C.

given solution of the acid does not change the concentration of the 1:1 chelate, and such additions have been shown to have little or no influence on the germination rate.

Germination with the 1:1 chelate of calcium and dipicolinic acid took place readily over a pH range of 5 to 9, but was generally most rapid at values close to pH 7. The concentration of calcium and dipicolinic acid required for rapid germination was between 20 and 40 mmole, which is somewhat higher for the aerobes we have tested than for the anaerobes. The optimum temperature for germination with the 1:1 chelate of calcium and dipicolinic acid was about 45°C for clostridial spores, but most aerobic spores were found to germinate very slowly or not at all at temperatures above 35°C. This was apparently because of the formation of a precipitate of calcium and dipicolinic acid when the concentration of calcium and the acid was higher than about 20 mmole. The rate of precipitation was increased at higher temperatures and

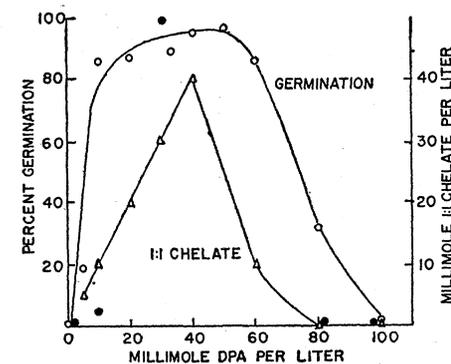


Fig. 1. Germination of spores suspended in 10 mmole of tris buffer, 40 mmole of CaCl₂, and varying concentrations of dipicolinic acid (DPA). Open circles: spores of putrefactive anaerobe S₂ incubated at 35°C for 60 minutes. Closed circles: spores of *Bacillus megaterium* incubated at 25°C for 30 minutes. Triangles: the calculated concentration of the 1:1 chelate of calcium and dipicolinic acid.