Reports

Inorganic Precipitation of Carbon in Productivity Experiments Utilizing Carbon-14

Abstract. In a series of experiments where iron was added to natural waters and photosynthesis was measured by the radiocarbon method, we found that inorganic precipitation of carbon-14, presumably by mechanisms of occlusive coprecipitation, resulted from additions of 100 or more micrograms of iron per liter. Low levels of iron and prefiltered controls are suggested as correction measures.

The use of carbon-14 for measuring the rate of carbon fixation has provided an extremely useful tool for estimating the primary productivity and limiting factors in natural waters. Because inorganic precipitation of carbon-14 can easily be misinterpreted as biological uptake, the following remarks serve as a cautionary note on the design and interpretation of experiments utilizing the high sensitivity of the carbon-14 method.

In assays (*1*) of Lake Tahoe, California-Nevada, for factors limiting photosynthetic carbon uptake, an increased activity was obtained from cultures of the natural phytoplankton population to which a trace-element mixture had been added. By adding elements singly and in combination it was determined that the increased carbon fixation was achieved with additions of iron as ferric citrate or ferric chloride. From considerations of the solubility product of the Tahoe euphotic zone, with an average *p*H of 7.0, one would expect 18 parts of soluble ferric iron per billion (ppb) (*2*). Actual analysis showed fewer than 10 ppb. Organic chelation would be minimal in this extremely oligotrophic lake. These data, and findings in the area of algal nutrition (*3*), suggested an iron deficiency in the lake, similar to that reported in Blind Lake, Michigan (*4*), and in the Sargasso Sea (*5*).

To find what level of iron was optimal for the stimulation of photosynthetic activity, additions of ferric chloride were made so that the cultures contained from 0 to 1000 ppb of added iron. Dark and light controls with the organisms removed by filtration through an HA Millipore filter were included because we had begun to suspect that at higher levels added iron might be exercising a nonbiological influence. Iron was added to these filtered controls at the 1000 ppb level.

There is apparent "stimulation" in the light at the 1000 ppb iron level when unfiltered water is used (Table 1). However, high values also occur in the dark and in the filtered cultures, suggesting that the "stimulation" is not entirely dependent upon the particulate, biological fraction of the water. Since the results obtained with unfiltered samples minus those obtained with filtered samples do not equal the measured activity of the control samples, biological inhibition is indicated at the 1000 ppb level. On examination of these samples, we found a ferruginous precipitate on all filters from cultures to which iron had been added. The amount of precipitate appeared to vary directly with the level of added iron. Comparable results were obtained in studies of sea water from the California coast and, recently, in studies of water from two antarctic lakes.

Increased carbon uptake in unfiltered Tahoe water was obtained with 10, 100, and 1000 ppb iron added as ferric chloride, with 1000 ppb added as ferric citrate, and in the presence of the chelating agent ethylenediamine tetraacetic acid in molar ratio of 10:1 (Fe:EDTA) (Table 2). In more thoroughly chelated form (molar ratio 1:1) the effect disappeared.

Clearly, abiotic precipitation of iron, presumably as hydrated ferric oxide, was occurring, especially at the higher levels of iron addition. The increased radioactivity of these filters can be explained by coprecipitation which may involve adsorption, occlusion, or postprecipitation of the carbon-14 with the colloidal iron. Anionic contamination, when colloidal ferric oxide is being produced in an aqueous system, has long been recognized by analytical chemists (*6*).

If the carbonate is primarily adsorbed to the surface of the iron particles, acid treatment should remove the greater part of it. This is the rationale for acid treatments of filters bearing C^{14}-labeled planktonic algae (reviewed in *7*). Filters from 1000 ppb iron cultures were successively treated with 10 ml of 0.003*N* HCl, 10 ml of 0.01*N* HCl, and 15 minutes in the vapors of fuming HCl. The loss of radioactivity checked at each stage never exceeded 6 percent of the initial count rate. The average loss of activity for eight filters was 3.2 percent after the three treatments. This strongly suggests that the carbon is tightly bound to the iron precipitate and that surface adsorption is not the primary means of binding.

Table 1. Assay of Lake Tahoe with added C^{14} and various amounts of added iron; values are counts per second corrected for machine and sample background.

Iron added (ppb)	C^{14} activity (count/sec)	
	Light	Dark
	Unfiltered water	
0	0.87	0.77
5	0.87	0.71
100	0.84	0.82
1000	1.24	1.18
	Filtered water	
1000	1.12	1.19

Table 2. Assay of Lake Tahoe with added C^{14} and various amounts of added iron in several forms; values are counts per second corrected for machine and sample background. The activity of the control (no added iron) was 2.97.

Iron added (ppb)	C^{14} activity (count/sec)			
	$FeCl_3$	Fe citrate	Fe (as $FeCl_3$): EDTA	
			1:1	10:1
1	2.95	2.78	2.89	2.91
10	3.01		2.58	3.11
100	3.43	2.74	2.85	
1000	4.25	3.78	2.80	4.63

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to contributors" [*Science* **125**, 16 (1957)].

No evidence of postprecipitation could be found in 22 cultures subsampled four times over a 48-hour period, although the initial subsample was not taken until 3 hours after the iron was added. One would also suspect, although the mechanisms of postprecipitation are not fully understood (*8*), that the carbon so precipitated would be localized peripherally on the iron colloids and hence be vulnerable to acid rinses. One might therefore conclude that the carbon is occluded within the iron oxide colloid where it cannot be removed by conventional washing procedures.

These results indicate the importance of preventing inorganic precipitation in experiments in which a radioactive carbonate tracer is used. It should be emphasized that precipitation may result not only from additions of nutrients, but also from *p*H shifts accompanying photosynthesis in closed bottles, from temperature and redox potential changes which can develop during prolonged incubation, from changes in the solubility of iron under the influence of light (*9*), and from particulate matter serving as precipitation nuclei. The addition of the basic solution containing carbon-14 itself, in some circumstances, could alter the *p*H enough to cause inorganic precipitation in natural waters. Dark bottles alone may not serve as adequate controls. We therefore recommend the inclusion of both light and dark prefiltered controls for all culture and productivity studies in which carbon-14 is used where inorganic precipitation may occur (*10*).

CHARLES R. GOLDMAN
DAVID T. MASON

Department of Zoology,
University of California, Davis

References and Notes

1. Methods, unless otherwise stated, are as described in C. R. Goldman, *Ecol. Monographs* **30**, 207 (1960); C. R. Goldman, *Ecology* **42**, 282 (1961).
2. W. Stumm and G. F. Lee, *Schweiz. Z. Hydrol.* **22**, 295 (1960).
3. W. Rodhe, *Symbolae Botan. Upsalienses* **10**, 149 (1948); G. C. Gerloff and F. Skoog, *Ecology* **38**, 551 (1957).
4. C. L. Schelske, *Science* **136**, 45 (1962).
5. D. W. Menzel and J. H. Ryther, *Deep-Sea Research* **7**, 276 (1961).
6. H. H. Willard and N. H. Furman, *Elementary Quantitative Analysis: Theory and Practice* (Van Nostrand, New York, ed. 3, 1940).
7. C. R. Goldman, "Symposia on marine productivity in the Pacific, 10th Pacific Science Congress, Honolulu, 1962," in press.
8. G. L. Clark, L. K. Nash, R. B Fischer, *Quantitative Chemical Analysis* (Saunders, Philadelphia, 1949).
9. S. I. Peltz and E. V. Lynn, *J. Am. Pharm. Assoc. Sci. Ed.* **27**, 774 (1938).
10. This work was supported by grants G-8901 and G-18020 from the National Science Foundation.

27 December 1961

Histoplasma capsulatum Isolated from Feather Pillow Associated with Histoplasmosis in an Infant

Abstract. Feathers from an old baby pillow yielded *Histoplasma capsulatum* in an epidemiologic study of histoplasmosis in an infant. Efforts to exclude other possible sources of infection, including blood donor and placental transmission, were exhaustive. Although histoplasmosis has long been associated with soils in avian habitats, the feather itself has not been implicated heretofore.

The best documented epidemiologic studies of histoplasmosis, whether in outbreaks or in sporadic cases, reveal the source of infection in nature to be soils in and around the habitats of domesticated and undomesticated avian species (*1–4*). The presence of *Histoplasma capsulatum* in such foci has been generally attributed to the high nitrogenous content of the soils, which presumably results from prolonged fertilization with bird or fowl excreta. This report describes the isolation of *H. capsulatum* from chicken feathers in a baby pillow more than 30 years old, which in an extensive epidemiologic investigation of a single case of clinical disease in an infant, 3½ months old, was the only source in the environment from which the organism could be recovered. The feather pillow has not been recognized before as a possible source of this infection.

The diagnosis of histoplasmosis in this infant was confirmed by isolation of *H. capsulatum* from his spleen after its removal, because of continuing enlargement, when he was 3½ months old. Because the baby was erythroblastotic at birth, it had to be presumed that the baby could have acquired the disease by direct transmission either from one of three blood donors or by the placental route, as well as by exposure to soils contaminated with the agent. The most usual means, exposure to contaminated soil, seemed the most unlikely, for the earth was blanketed with snow from the time the infant was discharged from the hospital at the age of 2 weeks until he was readmitted 3 months later. Nevertheless, when all members of the baby's family, including his mother, the three blood donors, and the family's pet dog proved by skin test and serology to be negative for histoplasmosis (*5*), soil samples and other types of specimens including several from potted house plants, were collected from the child's home environment. *Histoplasma capsulatum* was not isolated from any of these samples after injection into mice as described by Emmons (*1*). When it appeared that the baby had not acquired his infection in any of these three most probable ways, the baby's feather pillow was also investigated.

The small pillow, approximately 8 by 12 by 2 inches and covered with heavy muslin, was immersed and tumbled for a few minutes in sterile distilled water. Four specimens were prepared for intraperitoneal injection of 0.5 ml quantities into white albino mice weighing 14 to 16 g. Three specimens were sediments from surface rinse water collected after 30 minutes, and after 48 and 96 hours at room temperature. Each sample of 100 ml of rinse water was centrifuged to 5000 rev/min for 20 minutes and the sediment was resuspended in 10 ml of 0.85 percent sodium chloride solution containing 100 units of penicillin and 200 units of streptomycin per milliliter. The fourth specimen comprised washings from feathers removed from the case after 96 hours, by which time the case and feathers had begun to decompose. The feathers were

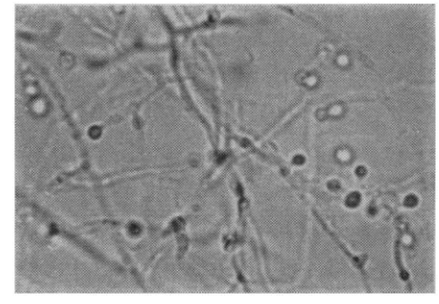

Fig. 1. Smooth-walled, round-to-pyriform conidia found in isolate from feathers after repeated mouse passage and one transfer on potato dextrose agar (about × 530).

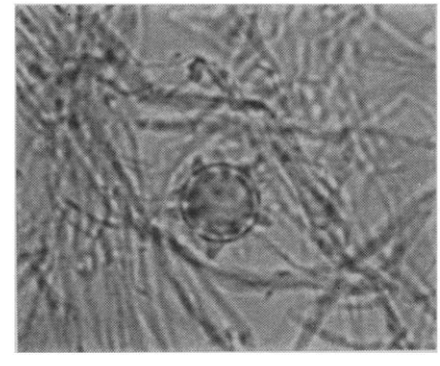

Fig. 2. "Macroconidium" characteristic of those observed in isolates of feathers after repeated mouse passage and several transfers on potato dextrose agar (about × 680).