types: for Japanese families of our earlier reports (*1*, *3*), the mean number of O children was 1.841 ± 0.187 for the ♀O × ♂B matings, 1.143 ± 0.175 for the ♀B × ♂O matings.

However, I do not intend to continue this discussion, because recently I recognized, in the course of analysis, that there was a possible bias in the record now available of Matsunaga and Itoh which, I suspect, had been introduced by the accidental loss (as mentioned in our earlier report) of a part of their original records.

In general I agree that there are many points left to be criticized in our analysis, but I believe that the possibility of prezygotic selection still exists, and that this must be tested in a refined way with unbiased, complete family data.

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2 January 1963

Fractionation of Sulfur and Carbon Isotopes in a Meromictic Lake

Abstract. *In the permanently stagnant depths of Green Lake (near Syracuse, N.Y.), sulfide made by bacteria is depleted in heavy sulfur (S^{34}), and sulfate is enriched. The fractionation factor, 1.0575, is the greatest yet observed. Isotopic resemblance to salt-dome sulfur deposits is evident, and, like salt-dome calcite, the lake's carbon dioxide is depleted in heavy carbon (C^{13}).*

Anaerobic bacterial oxidoreductions, characteristic in lake mud but also detectable in deep water during seasonal stagnation, ferment organic carbon to CH_4 and CO_2 and reduce sulfate to sulfide. The anaerobic fraction of a lake's total metabolism is likely to be large in a small lake or in a lake with a reducing hypolimnion. Isotopic fractionation by biochemical reactions identifies effectively the products of this metabolism. For example, in Linsley Pond, Branford and North Branford, Conn. (*1*), where the organic substrate (dead plankton and fresh mud) has a heavy carbon content, ϑC^{13} (*2*), of −30 per mil, the CH_4 of mud gases is depleted in C^{13} to −77 per mil, while the CO_2 is enriched to −4 per mil. That is, we observe in nature a fractionation of C^{13} by a factor of 1.073, nearly as large as the 1.094 found in culture (*3*) when methane bacteria ferment methyl alcohol. As one consequence, the net C^{13} content of the hypolimnion varies with the proportion of the fermentative (C^{13}-enriched) source and is ordinarily quite different from that of surface waters. Sulfur metabolism is equally amenable to this kind of tracer geochemistry; in the same lake (*4*), although H_2S rarely appears in the water (owing to high concentrations of Fe^{++}), we infer its formation from the enrichment of heavy sulfur (S^{34}) in the sulfate that remains in solution after preferential removal of S^{32} to form sulfide. This observation provides the first direct proof that bacterial reduction affects the sulfate in lake water (*5*).

Table 1. Limnological and isotopic data from Green Lake, Fayetteville, N.Y., 30 June 1962.

Depth (m)	Temp. (°C)	O_2 (mg/lit.)	Sulfur (mg/lit.)		ϑ S^{34} (per mil)		CO_2 ml/lit.)	ϑ C^{13} (*11*) (per mil)
			SO_4^{--}	S^{--}	SO_4^{--} (*6*)	S^{--} (*2*)		
0	21.52	5.97	446	0.0	+23.7		55.7	− 7.0
18	9.35	5.50	449	0.0	+24.4		109.8	−11.2
20	7.91	2.70	455	6.7	+25.3		125.9	−13.7
25	7.68	0.65	446	18.4	+27.5	−28.8	144.8	−17.0
35	7.70	0.0	444	25.4	+28.2	−26.8	147.9	−16.0
45	7.85	0.0	417	34.3	+31.3	−26.3	166.1	−17.8
52	7.90	0.0	422	39.4	+30.0	−25.0	164.3	−20.1
Inlet (*11*)			298	0.0	+24.7			

The maximal S^{34} fractionation so far observed in Linsley Pond is 20 per mil, or 1.020; the sulfide-rich mud has $\vartheta S^{34} = -7$ per mil (*6*), while ϑS^{34} in deep-water sulfate rises to +13 per mil in late summer. Considerably greater fractionation is known (*7*), however, in bacterial cultures, especially when rates of sulfur metabolism are kept low. Kaplan *et al.* found fractionation factors as large as 1.046 (*8*). Greater fractionation than this has not been found in any single system, and when geochemists encounter unusual enrichment or depletion they automatically invoke some special situation, such as a multistage or steady-state process.

Suspecting that S^{34} fractionation might be enhanced by permanent stagnation, we visited the nearest known example of meromixis, Green Lake, at Fayetteville, near Syracuse, N.Y. This lake, known to geologists (*9*) for its massive fringing reefs of algal limestone, occupies the plunge basin of a late-glacial waterfall, and is meromictic primarily because of its great depth (59 m) and small area (27.08 ha). In 1961, as Eggleton (*10*) found in 1925–1939, the stagnant monimolimnion lay below 20 m, constituting nearly half (47.5 percent) of the volume, and contained more than 30 mg of sulfur per liter as H_2S. The water at 20 m is bright magenta; Taylor identified this for Eggleton as the purple sulfur bacterium *Lamprocystis roseopersicina* Cohn. Unusual quantities of sulfate and carbonate, derived from sedimentary rocks that evidently include some gypsum, maintain the stable stratification today and may have helped initiate it.

The H_2S and the SO_4^{--} show a great difference in S^{34} content (Table 1). The fractionation factor, 1.0575, is so much larger than expected that the possibility of some multistage process must be considered. The following facts are relevant:

1) The S^{34} ratio of inlet water is +24.7 per mil, and that of the surface of Round Lake, ¼ mile upstream, is +25.6 per mil (*11*); these values exceed that of seawater and indicate gypsum or anhydrite as the probable source of sulfate. In the lake, deep-water sulfate is moderately enriched in S^{34} by 6.7 per mil over the sulfate in the inlet, while the surface waters are actually depleted by 1 per mil.

2) The sulfate content of the inlet is 296 mg of sulfur per liter, and that of Round Lake is 319 mg/liter, which is very high for inland waters, but lower than the 441 mg/liter of Green Lake itself. Though the possibility of deep gypsiferous springs cannot be excluded, we would rather expect a meromictic lake to be a trap for sulfur.

3) The mixolimnion and the monimolimnion are only approximately in isotopic balance; there is more sulfur, and proportionately more S^{34}, at all levels below 20 m than in the mixed surface waters.

4) There is so little sulfur in the bottom deposits that we have been unable to measure it. If H_2S is mainly made in the water and is not precipitated as FeS, as it is in Linsley Pond,

it can diffuse to the overlying mixolimnion.

5) The steady upward diminution in the S^{34} content of the sulfate suggests that some (S^{32}-rich) sulfide is being reoxidized where O_2 is present. Such relatively light sulfur is in a position to be lost by the outlet.

The model implied by these data is a reflux system with a leak near the top. Net accumulation of sulfur, if real, must occur through sedimentation of plankton or by addition from springs having more sulfate than the inlet; in either case the S^{34} content would be about the same as that of the inlet. In the absence of iron, any loss of sulfur from the deep-water trap is by volatilization, and as the light H_2S diffuses upward and is reoxidized to sulfate without fractionation, the outlet, in removing some surface water, removes S^{32} preferentially.

This mechanism will explain the enrichment of the monimolimnion in S^{34}, but it will not explain an abnormally large fractionation observed *in situ*. If the maximal fractionation between sulfate and sulfide is 46 per mil, whatever the ratio in the parent sulfate, this amount, and not 57.5 per mil, should be the difference found. We see no reason to postulate a multistage sulfur-redox system within Green Lake; fractionation by 1.0575 evidently can occur in a single step, ecologically, if not biochemically.

The anhydrite associated with Gulf Coast salt domes is notably enriched in S^{34} (*12*) by a closely similar process, that is, by bacterial removal of some of its S^{32} to form native sulfur. As sulfur bacteria require organic carbon, which normally has a C^{13} content of about −30 per mil, bacterial origin of the sulfur is confirmed by the fact that $CaCO_3$ formed in the same environment contains less C^{13}, by about 30 per mil, than other limestones. For the same reasons, C^{13} ratios as low as −33 per mil are found (*13*) in the Sulfur Limestones of Sicily.

As Table 1 shows, Green Lake is comparable to a salt-dome system in respect to its carbon as well as its sulfur isotopes. Some depletion of C^{13}, quite unexpected in a lake so rich in carbonate, is evident even in the mixolimnion. Still greater depletion in the monimolimnion (to $\vartheta C^{13} = -20.1$ per mil) demonstrates massive admixture from some C^{13}-poor source, presumably organic. As we have no definite basis for distinguishing aerobic from anaerobic CO_2, or for recognizing the initial endowment of HCO_3^-, we cannot explain these results in detail. We presume, however, that the production of CO_2 from organic matter by sulfate-reducing bacteria, though a special type of fermentation, does not enrich the CO_2 in C^{13}, and may even deplete it. We have also to remember that at the top of the monimolimnion some H_2S is being oxidized to S_2, and probably to SO_4^{--} as well, by bacterial photosynthesis, with unpredictable consequences for the distribution of carbon isotopes.

When such questions are settled we hope to take advantage of the coupling of carbon fixation to sulfate reduction, and calculate rates of metabolism, and the time of onset of meromixis, by geochemical stoichiometry. Meanwhile, we point to Green Lake as isotopically labelled "queer" and wonder how many geologic records of meromictic lakes may be contributing to the confusion of geochemists (*14*).

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$$\vartheta C^{13} = \frac{C^{13}/C^{12}{}_{(spl)} - C^{13}/C^{12}{}_{(std)}}{C^{13}/C^{12}{}_{(std)}} \times 1000$$

where *spl* is the sample and *std* is the standard.
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6 November 1962

Cholinergic Action of Homogenates of Sea Urchin Pedicellariae

Abstract. *A dialyzable, acetylcholine-like material exists in homogenates of globiferous pedicellariae of the sea urchin* Lytechinus variegatus. *Its pharmacological characterization was obtained on the basis of the responses of the guinea pig ileum, the rat uterus, blood pressure in the dog, the amphibian heart, the longitudinal muscle of the holothurian, and the protractor muscle of the sea urchin lantern. Some of the data were statistically treated, and an attempt was made to determine a ratio of potency between the homogenates and acetylcholine.*

Little is known about the nature of the substance (or substances) involved in the toxic action of the pedicellariae of the sea urchin. Extracts of all four types of pedicellariae are toxic when injected, paralyzing such animals as crabs, fishes, and even lizards, but frogs and other echinoderms are reported to be more or less immune (*1*). The extract of the globiferous pedicellariae is the most effective. Fujiwara (*2*) reported that "bites" into his finger of seven or eight globiferous pedicellariae of *Toxopneustes pileolus* evoked severe pain, followed later by dizziness, some facial paralysis, and difficulty in breathing. It seems, however, that no attempt to study the problem pharmacologically has yet been made.

In the course of a study of the responses of the protractor muscles of the sea urchin lantern to drugs and crude tissue preparations, we observed that they were sensitive to extracts of pedicellariae. The fact that these muscles are only known to respond to acetylcholine and some other choline deivatives (c) led us to make a broader investigation of the effect observed with the extracts of pedicallariae.

Large globiferous pedicellariae, which occur abundantly on the aboral surface of the toxopneustid *Lytechinus variegatus* (*4*), were used in the experiments. They were picked up with fine forceps, blotted in filter paper, and weighed to obtain 1:100 dilutions of stock homogenates. Homogenization took place in filtered sea water (for marine animals) or saline (Ringer or Tyrode solution), and when necessary the *p*H was adjusted to 7.0. Dilutions of the stock homogenates were tested against effector systems long recognized as cholinergic (such as the guinea pig