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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$$\vartheta S^{34} = \frac{S^{34}/S^{32}_{(spl)} - S^{34}/^{32}_{(std)}}{S^{34}/S^{32}_{(std)}} \times 1000$$
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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

ileum and the amphibian heart) or recently reported to be cholinergic (the protractor muscle of the sea urchin lantern and the longitudinal muscle of the holothurian).

The longitudinal muscle of *Holothuria grisea,* which is extremely sensitive to acetylcholine (*5*), first responded to 0.5 μg of the pedicellariae extract (in a 15-ml bath). The protractor muscle of the lantern of the sea urchin *Echinometra locunter* is less sensitive to acetylcholine (*3*). It first responded to 0.1 mg of the pedicellariae extract (in a 10-ml bath). The protractor muscle behaves in pharmacological tests like the ileum of the guinea pig (*3*), quickly returning to base line after washing out of the drugs. This permitted us to test the linearity of the curve relating response and dose (expressed on a log scale), using four groups of three doses (0.4, 0.8, and 1.6 mg). The statistical treatment of the data produced a regression line given by the equation:

$$Y = 28.75 + 15.26\ (x - 0.93)$$

The ratio of variances ($F = 601.50$) for the regression line was far above the 99.9-percent level of probability. Deviation from regression, however, was a little above the value for experimental error, although the standard error represented less than 10 percent of the mean for all experiments. This action of pedicellariae homogenates on the protractor muscle is blocked by both atropine and Mytolon, as observed for acetylcholine in this long-fibered smooth muscle (*3*).

The heart of the toad *Bufo ictericus* reacts to 0.1 mg of the pedicellariae homogenate by a slowing down of the rhythm. Treatment with 1 mg induces a diastolic block; this can be prevented by previously atropinizing the heart.

In the case of the guinea pig ileum, we first checked to find whether or not the pedicellariae homogenates had a histaminic action. In a 10-ml bath, 1 μg of Benadryl (which has some atropinic action) totally abolished the response to 0.1 μg of histamine and depressed by about 33 percent the action of 0.8 mg of the pedicellariae homogenate. On washing out of the Benadryl, the recovery of sensibility of the gut to the pedicellariae extract was almost immediate. Treatment with 0.005 μg of Neo-antergan (which has practically no atropinic activity) significantly reduced the response of the ileum to 0.1 μg of histamine but had no effect on the response to 0.8 mg of the pedicellariae extract. Treatment with 0.1 μg of atropine completely abolished the response of the ileum to 0.3 mg of the pedicellariae extract, and a gradual recovery of sensibility was observed on washing out of the drug. Treatment with 0.2 μg of Prostigmin or 0.2 μg of eserine about doubled the response of the gut to 0.2 mg of the pedicellariae homogenate.

To test the linearity of the curve relating the dose and the response of the gut in a log scale, three doses of the pedicellariae extract (0.4, 0.8, and 1.6 mg) were added in four groups. After the slope had been estimated ($b = 27.5$), the regression line was given by the equation

$$Y = 34.0 + 27.5\ (x - 0.8)$$

The ratio of variances ($F = 493.56$) for the regression line was far above the 99.9-percent level of probability. Deviation from regression was below the experimental error, and the standard error of the assay represented less than 10 percent of the mean for all determinations.

The acetylcholine-like material contained in the homogenates is dialyzable and partially inactivated by heating. Complete inactivation was obtained by treatment for 1 to 2 hours with $N/4$ NaOH, followed by neutralization with HCl.

Experiments conducted with the help of D. Valente and G. Maugé showed that the pedicellariae homogenates depress blood pressure in the dog and act on the uterus of the rat.

Thus, the results indicate the presence, in the globiferous pedicellariae of *Lytechinus variegatus,* of a material with many of the characteristics of acetylcholine. On the assumption that it is possibly acetylcholine or a nearly related compound, a four-point assay (*6*) for the ileum of the guinea pig was performed in order to determine the ratio of potency between a standard acetylcholine solution and an unknown pedicellariae homogenate. The doses chosen were within the range of those producing small to submaximal contractions. In the assay, taking doses of the standard (s) and the unknown (u) in the ratio 1:4 (0.1 and 0.4 μg of acetylcholine; 0.1 and 0.4 mg of the pedicellariae extract) and randomizing the doses in five groups of four, we obtained results as follows: $M = U/S = 1.21$; confidence limits, 1.32 and 1.12; real ratio of potency, 1.15.

The parallelism, however, was not strict, perhaps because the homogenate used was crude. But even so the method can afford a reliable indication of the ratio of potency (*7*).

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20 November 1962

Erythrocyte Acid Phosphomonoesterase and Glucose-6-Phosphate Dehydrogenase Deficiency in Caucasians

Abstract. *Caucasian patients with erythrocyte glucose-6-phosphate dehydrogenase deficiency also have a deficiency in erythrocyte acid phosphomonoesterase. This acid phosphomonoesterase deficiency is not present in Negroes with the glucose-6-phosphate dehydrogenase deficiency.*

Deficiency of glucose-6-phosphate dehydrogenase (G-6-PD) in erythrocytes is now a widely recognized hereditary defect which occurs in both Negroes and Caucasians. Accumulating clinical evidence suggests that there may be biochemical differences between enzyme-deficient patients in these two racial groups. For example, there is no report of the occurrence of congenital nonspherocytic anemia in Negroes with G-6-PD deficiency although there are several references to this condition in enzyme-deficient Caucasians (*1*). Also, the ingestion of fava beans by enzyme-deficient Negroes has not been found to result in acute intravascular hemolysis as it does in Caucasians with a G-6-PD deficiency (*2*).

In an attempt to demonstrate enzy-