## "Polluted" Water from the Leaching of Igneous Rock<sup>1</sup>

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Unpolluted surface and ground waters in the uplands of northern Georgia have been found to contain substantial concentrations of fixed nitrogen. Since nitrogen concentration has long been used as an index of water pollution, the observation of concentrations of 1.5-2.0 ppm in a small mountain stream at a point 2500 ft above sea level aroused our curiosity. After some study, we believe that this and numerous other observed occurrences of fixed nitrogen in presumably unpolluted waters of the area can be attributed to leaching of soluble nitrogen compounds from granite during weathering. A brief account of the investigation leading to this conclusion is presented here. in the fog of volcanoes, it appears that it is fairly abundant in the interior of the earth. Thus, where the interior rocks now appear at the surface, ammonium chloride could have been included in them at the time they were formed.

The authors considered the hypothesis that the rapid rate of weathering of the granite rock in this area can be explained by leaching of trace amounts of soluble substances such as ammonium carbonate or ammonium chloride. With this in mind, pieces of igneous rock that had weathered both rapidly and slowly were brought into the laboratory, crushed, and then leached with a fairly large amount of water. The leachings were analyzed for the ions of ammonia, calcium, magnesium, chloride, nitrate, and carbonate. An attempt was made to determine whether soluble substances were present in higher amounts in rocks that weathered rapidly.

Results of a number of analyses of the leachings (Table 1) indicate that many igneous rocks yield

| Sample                     | Location           | $\mathbf{pH}$ | Carbonate<br>system<br>(mg/100 g) | Nitrogen<br>(mg/100 g) | Chloride<br>(mg/100 g) | Calcium<br>plus<br>magnesium<br>(mg/100 g) |
|----------------------------|--------------------|---------------|-----------------------------------|------------------------|------------------------|--|
| Hard unweathered           | Clayton, Ga.       | 8.0           | 20                                | 1.5                    | 15                     | 10   |
| Partially weathered        | ° ( ´ ( (          | 7.8           | 18                                | 1.3                    | 10                     | . 10                                       |
| Decomposed granite         |                    | 8.2           | 15                                | 1.2                    | 12                     | 8  |
| Granite-gneiss             | Toccoa, Ga.        | 6.3           | 22                                | 1.5                    | 25                     | 12   |
| Fresh basalt               | Hawaiian Islands   | 6.8           | 420                               | 2.3                    | 70                     | <b>64</b>                                  |
| Unweathered granite-gneiss | Lithonia, Ga.      | 9.3           | 72                                | 1.8                    | 58                     | 30   |
| '' Granite                 | Mount Airy, N. C.  | 9.3           | 95                                | 5.4                    | <b>50</b>              | 25   |
| "                          | Isle, Minn.        | 9.4           | 122                               | 2.0                    | <b>4</b> 0             | 80   |
| <b>«</b> «                 | Coneste, S. C.     | 8.0           | 40                                | 8                      | 14                     | 84   |
| " "                        | Atlanta, Ga.*      | 9.0           | 124                               | 2.8                    | 4                      | 64   |
| " "                        | ·· ·· <del>†</del> | 8.3           | 7                                 | 1.8                    | <b>54</b>              | 18   |

\* Hemphill Quarry on Northside Drive.

† Moreland Avenue, Southeast.

A series of analyses of well waters in the Atlanta area showed that many of them were extremely high in nitrogen as either ammonia, nitrite, or nitrate. One flowing spring under a large hotel in the city contained 40 ppm nitrate nitrogen. However, high nitrogen values in the city could possibly be explained by the use of lawn fertilizers or the presence of packing houses or dairy barns. On the other hand, when a small mountain stream and a spring that is tributary to it showed a nitrogen content of 1.5 ppm, it was evident that some natural cause might be responsible. The high value of nitrogen in the stream is approximately 100 times the nitrogen value for good drinking water.

A search of the geological literature did not disclose why the stream and well waters in northern Georgia should contain such high nitrogen values. However, since ammonium chloride is a commonly dispersed gas soluble substances in approximately the same amount. Although some rocks weather at much more rapid rates than others, the rate of weathering of the rocks did not correlate with the fixed nitrogen value. Furthermore, an analysis of the leachings from some deeply weathered granite from northern Georgia near Clayton showed that the weathered residue still contained rather large amounts of easily leached ammonia. This fact is not well understood because, even though the rapid rate of weathering explains the high value for the ammonium chloride in the leached solution, it would be expected that the ammonium chloride should leave the rock during the early stages of weathering. However granite-gneiss also shows a high ammonium content, and yet it weathers very slowly.

The next question to be answered was the source of the ammonium chloride. It was possible that the nitrogen had been fixed from the atmosphere by the biological agencies in leguminous plants and then intruded into the rock from the ground water. A fresh sample of basalt (from 13,000 ft above sea level) from

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the recent eruption of Mauna Loa in the Hawaiian Islands was obtained from the territorial geologist. When this sample was crushed and leached, it showed a nitrogen content that is typical of the rocks that were found in the Georgia area. It appears, therefore, that the nitrogen content of Georgia granite is typical of other igneous rocks. It is not surprising that the nitrogen content of granite has not been studied, because its content at 25 ppm is certainly lower than the normal analytical procedures of geochemistry would detect. Furthermore, nitrogen is not picked up on a spectrochemical analysis in the excited electric arc; therefore, the discovery reported in this paper was made possible only because of the use of solution chemistry from the rock rather than by the use of **a** direct analytical approach. This type of chemistry has led to important developments in the use of granite dust, which, when applied to land very low in vegetation because of lack of the soluble substances needed for plant growth, has transformed it into a rich and verdant area.<sup>3</sup> The increase in plant growth has obviously resulted from the presence of the calcium, magnesium, potassium, sodium, and ammonia that have been leached from the dust by the action of rain water.

Previous sanitary analyses of the Chattahoochee River, which has a normal flow of something over 1000 cfs, have not shown any large nitrogen content. However, it should be remembered that the large flow includes a heavy surface runoff, which is not typical of a small mountain stream flowing slowly over and through residual soil.

The conclusion seems warranted, therefore, that the concentration of soluble compounds in spring water or surface water that has a chance to flow slowly over granite is determined by the rate of rock-weathering. The nitrogen content of waters in an area where there is rapid weathering of granite cannot be used to determine the load of human pollution, as is frequently done in areas where granite weathering is very slow.

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<sup>3</sup>Anon. Granite Dust Builds Better Soil. Org. Farmer, 3, 38 (Mar. 1952).

# Production of Better Penicillin-producing Strains by Mutation Induced by Uranium Nitrate

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Stakman, Daly, Gattani, and Wahl (1) have shown that addition of uranium nitrate to potato dextrose agar at the rate of 0.5–1.0 g/liter stimulated mutation in the cultivated mushroom *Agaricus campestris*, and both mutation and an unusual type of dissociation in the ordinary corn smut fungus, *Ustilago zeae*. They suggested that addition of uranium nitrate or other similar salts may be a simple and useful means of inducing desirable mutations in at least some microorganisms. The agar containing uranium nitrate is mildly radioactive, as determined by Alexander Hollaender, Oak Ridge National Laboratory (1). The present studies were undertaken with a view to obtaining some better penicillin-producing mutants from their parents by growing the fungus on media containing uranium nitrate.

Penicillium notatum chrysogenum strain 18, isolated by Gattani and Kaul (2) from Indian soil samples, and strain Minnesota X1612 were used for these studies. This strain was brought by the author from the Department of Plant Pathology, University of Minnesota, in 1946 and was stored in sterile sand at room temperatures in India.

The method followed for inducing mutants in these strains differed from that used by Stakman et al. The strains were first grown on potato dextrose agar medium containing 0.5 g uranium nitrate/liter. The actively growing mycelium was then transferred to media containing 1.0, 1.5, or 2.0 g uranium nitrate/ liter. By growing the fungus first on a low concentration of uranium nitrate and subsequently transferring the fungal mycelium to media containing higher concentrations of uranium nitrate, the frequency of mutation, as evinced by the production of morphologically distinct sectors, was enhanced two to three times. When the fungus mycelium was transferred directly from potato dextrose agar to media containing higher concentrations of uranium nitrate, the growth of the fungus was inhibited, and number of sectors produced was comparatively less.

Strain 18 produced mutants that could be broadly classified as white, entirely mycelial, nonsporeforming, or green mutants. Some of the white mutants spontaneously produced secondary green mutants. In Minnesota X1612 the mutants were of different shades of green, no white mutants being produced in this strain.

Penicillin-producing ability of the mutants and their parents was compared by the plug method, described by Raper, Alexander, and Coghill (3). One mutant, 18G, from more than 30 mutants of strain 18 tested, showed increased penicillin production; the four radial series plugs of this mutant strain produced bigger circles than those produced by the four plugs of the parent strain. Similarly, the four radial series plugs of one mutant of Minnesota X1612, designated as X1612-C, gave bigger circles of inhibition than those produced by the parent strain.

Unfortunately, because of inadequate facilities and nonavailability of corn-steep solids, the mutants of Minnesota X1612 and strain 18 could not be tested by measuring the amount of penicillin produced under submerged conditions on that medium. However, mutant strains and the parent strains were grown on Czapex liquid medium in surface culture. The culture filtrate was assayed after the 6th, 7th, 8th, 9th, and 10th days of inoculation. Invariably, 1:100 dilutions of the culture filtrate from the mutant strains 18G and X1612-C produced consistently bigger lytic zones than those produced by the parent strains 18 and X1612,