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.[3] 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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[3] 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,

TABLE 1

SIZE OF LYTIC ZONES PRODUCED BY THE PARENT STRAINS AND THEIR MUTANTS

Strain No.	Size of lytic zone in mm				
	6th day	7th day	8th day	9th day	10th day
Minnesota X1612	21	28	27.5	27	22
X1612-C	24	30.5	29.1	28.5	25
18	18	19	23.5	25	24
18G	19	20	25	27.2	26.3

respectively. This shows that they produced more penicillin than did their respective parents on Czapex Dox medium. Table 1 summarizes these results.

As a result of these investigations it appears that addition of uranium nitrate to nutrient media may be a simple means of inducing desirable mutations, with respect to penicillin-producing ability, in at least some strains of *P. notatum chrysogenum*.

References

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Culture of Cell Suspensions and some Results

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In regular tissue cultures, the embryo extract used as a constituent of the culture medium contains a great variety of growth-promoting substances and, in all probability, a number of growth-controlling factors. It is prepared, as a rule, from the clear supernatant obtained by the centrifugation of minced embryos. Sometimes, in cultures made up with freshly prepared extract, small groups of living cells are seen to grow without apparent connection with the main cell colony. These, it seems, originate from scattered cells that persist in the extract after centrifugation.

In an examination of this it has been found that in the centrifugation of minced embryos in the usual laboratory centrifuge, if no saline has been added, a clear supernatant is obtained only when the embryos are younger than 10 days. Ten- and 11-day-old embryos give an opaque, grey-white, viscous supernatant, and embryos older than 11 days under the same conditions of centrifugation give no supernatant in the usual sense at all. This opaque fluid contains a fine suspension of cells and may be used in culturing where the aim is to obtain diffuse cell growth without larger tissue explants.

Ten- or eleven-day-old chick embryos, thoroughly minced by small scissors, were centrifuged at a speed of approximately 2000 rpm for 20 min. The opaque viscous supernatant was spread in a film on a cover glass 30×30 mm, or larger, or placed in a culture flask. When the cover glass cultures were examined under the microscope, the suspension was seen to be composed of unconnected, apparently damaged, single cells and detrituslike cell splinters and a few isolated cell groups.

After 24–48 hr of incubation the microscopic picture had changed completely: Almost all isolated cells had become connected with one another by long, thin cell strands, or bridges, forming a continuous tissue network with meshes of various shapes and sizes (Figs. 1, 2). The growth area, which can be very large, depending on the area used for planting, showed variously differing cell clusters, distinguished by the preponderance of certain cell types, such as epithelial membranes, nerve cells, pigment cells, etc. When treated with a 1:40,000 solution of neutral red, all cells displayed normal vital staining. Frequently a culture would contain many single but normal-looking isolated cells which also showed normal vital granules. Some of those cells were seen in pairs as though adhering to one another after division. The most interesting feature of a suspension culture is the reticular growth which tends to connect, by filamentous long cell bridges, all cell groups into a continuous whole (Figs. 1, 2).

In a series of cultures, chicken plasma was added to the suspension on the cover glass, and clotting of the suspension was obtained. Growth of these cultures was likewise abundant, but the form of cell connections appeared strikingly different (Fig. 3). There was no visible reticulum. It would seem that differences in the physical character of the medium introduced by the plasma had had some influence on cell form and cell distribution. The difference displayed is one to be explored in a study of factors influencing tissue formation.

Suspension explants may also provide a means of approaching the problem of tissue reconstruction out of isolated cells. The following experiments illustrate this: Four parts of Ringer's solution was added to one part of the original cell suspension, thoroughly mixed, and centrifuged as indicated above. The now clear supernatant was removed, and the residue was again suspended in 4 parts of salt solution and centrifuged. This washing procedure was repeated 4 times, and thereupon the residue was suspended in Ringer's solution, from which suspension cultures were prepared. These cultures showed no signs of growth. In view of the fact that tissue fragments placed in Ringer's solution often are capable of producing cell growth in a regular tissue culture, the above result suggested that by the described washing procedure a substance might have been washed away which was responsible for the reticular tissue formation out of isolated cells. In support of this it was found that when various amounts of embryonic juice were then added to suspensions after having been washed as described, typical reticular tissue growth was obtained; this was the better the greater the concentration of embryonic juice