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*.

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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.

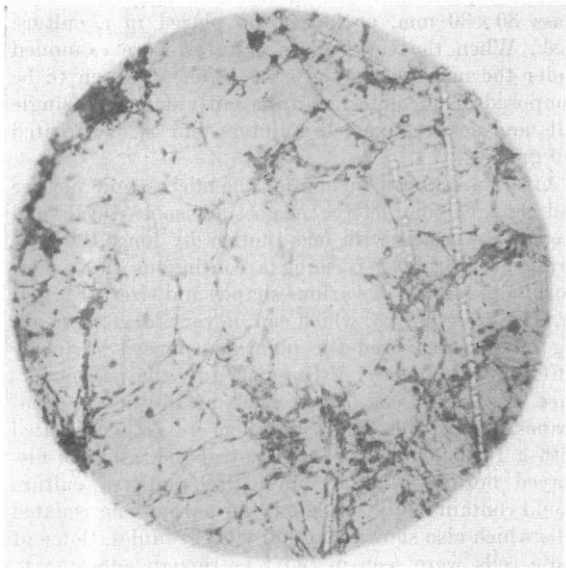
In an examination of this it has been found that in the centrifugation of minced embryos in the usual laboratory centrifuge, if no saline has been added, a clear supernatant is obtained only when the embryos are younger than 10 days. Ten- and 11-day-old embryos give an opaque, grey-white, viscous supernatant, 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.

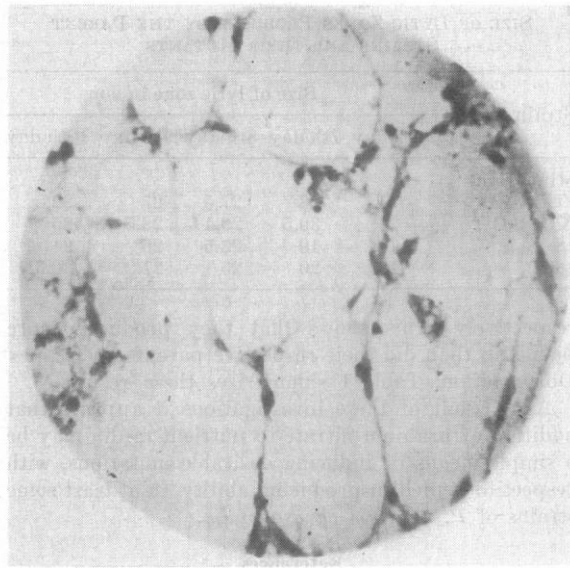
Ten- or eleven-day-old chick embryos, thoroughly minced by small scissors, were centrifuged at a speed of approximately 2000 rpm for 20 min. The opaque viscous supernatant was spread in a film on a cover glass 30×30 mm, or larger, or placed in a culture flask. When the cover glass cultures were examined 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





FIGS. 1 and 2. Cell suspension cultures. (Bouin ; hematoxylin-eosin.)

in the suspension culture. By far the best growth of suspension explants was obtained when the residue was suspended in pure embryonic juice.

Two observations from these experiments may be considered: (*a*) The failure of suspension explants to grow without embryonic juice, whereas in regular tissue culture cell growth in pure Ringer's solution can be obtained; and (*b*) the capacity of pure, concentrated embryo juice to promote the growth of these cell suspensions, whereas in regular tissue cultures such concentrations are known to be inhibitory (*1, 2*).

In commenting on the first of these, the conclusion seems justified that suspension cultures need a greater amount of a substance present in embryonic juice and different from growth substances proper. This substance would appear to be the limiting factor for reconstructing reticular tissue out of isolated cells in suspension explants.

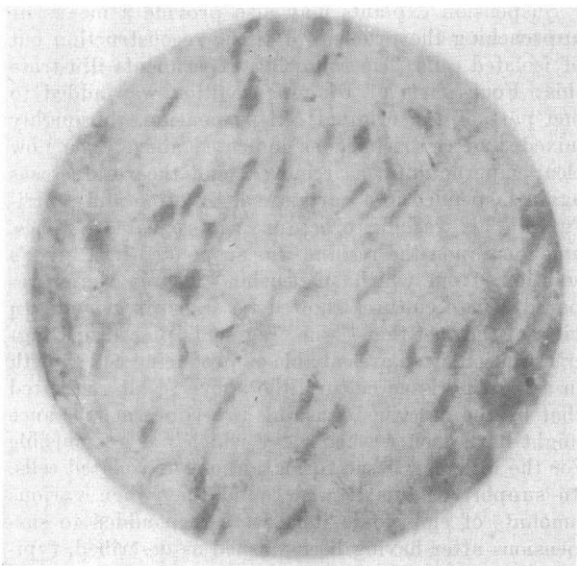


FIG. 3. Cell suspension culture grown in plasma clot. (Bouin ; hematoxylin-eosin.)

At one time consideration was given to the possibility that this substance might be mucinlike—i.e., that amino sugars might be responsible for maintaining the adhesiveness of cell borders, or, in particular, that hyaluronic acid might possibly be functional in the formation of connective tissue. This substance, it was thought, might have been washed away by our washing procedure and would have to be replaced by a concentrated embryonic juice containing sufficient amounts of it. In support of that concept it has been shown that suspensions prepared from embryos with their eyes removed gave less reticular growth than when whole embryos were used. Eye tissues, in particular the corpus vitreum, obviously are rich in hyaluronic acid. However, the role that mucinlike substances, such as hyaluronic acid, may play in the formation of reticular growth in suspension cultures is yet far from being established.

The second of these observations can be explained in a natural way by the assumption that embryonic juice, besides containing substances that promote cell reproduction, also contains substances that encourage tissue formation while at the same time inhibiting cell reproduction. As soon as the concentration of embryo juice surpasses the optimum for cell growth, the higher concentration of growth-inhibiting substances in the concentrated embryo juice may become effective.

Because the suspension consists of cells forcibly torn out of the organized tissue units, and the regenerative capacities tend to reconstruct a new tissuelike cell organization, this method may provide another way of approaching the problem of the relationship between

reproduction and tissue formation. It provides, as well, a form of explanation without a central, partly necrotic tissue fragment, from which undefined substances are released into the medium such as to render it less suitable for quantitative determinations of metabolic processes.

It has been found that explanted suspensions containing cells originating from different organs, and from different animals, can form a cell reticulum and tissue continuity. This method can therefore be used for studying problems of protoplasmic specificity. There is some reason to justify the assumption that the method will prove convenient in providing a substrate of tissue for the propagation of viruses.

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A New Criterion for Weathering in Soils[1]

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Weathering consists of the mechanical disintegration and chemical deterioration of minerals at or near the ground surface. In soils, this process usually results in the development of a profile that may be described in terms of A, B, and C or D horizons. The greatest weathering occurs in the surface horizons, with a gradual decrease in intensity downward toward the parent rock. This simple picture of a soil profile is essentially that of a monogenetic soil defined by Bryan and Albritton (*1*) as one that has matured through a relatively uniform climate. Polygenetic soils (*1*) are those that have matured through at least two time intervals of different climates, with profile types superimposed one on the other. Such soils are not easily distinguished in the field. In addition, glacial, alluvial, and eolian depositions are common sources of superimposed profiles of weathering.

Differentiating between monogenetic and polygenetic profiles and recognition of buried profiles require distinction of the various soil horizons on the basis of an objective weathering criterion. This paper proposes such a criterion.

Recent studies by the authors have revealed a positive criterion for differentiating weathering regimes in a soil profile. The basis for this criterion is x-ray examination of the clay and silt fractions of the various horizons in the soil profile. If examination reveals the presence of mica minerals in either of these size fractions, the means are at hand for identifying the sequence of weathering. As this mineral group occurs so frequently in soils, the method of diagnosis should prove generally useful to pedologists and geologists.

Mica weathering is essentially a process of potassium depletion. Vadose water actively enters into hydrolytic interplay with the interface K^+ of the mica lattice. In the usual acid medium near the ground surface, this activity results in the displacement of this cation, with a consequent loosening of the bonding effect between mica sheets. Brown (*2*) has suggested

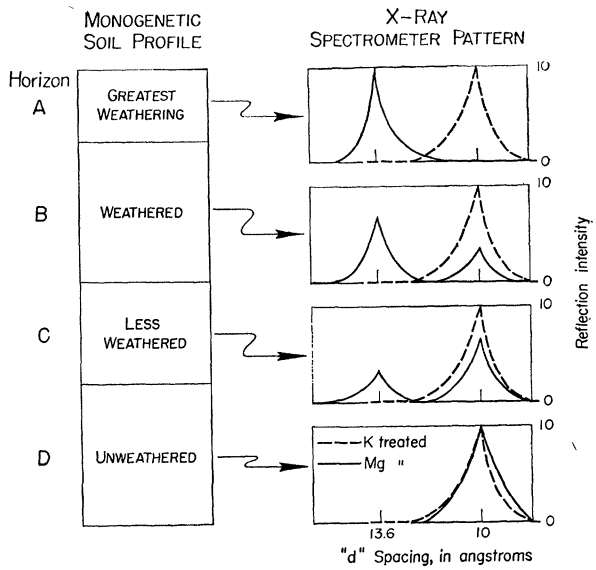

FIG. 1. Diagrammatic illustration of the weathering criterion.

the name "hydrous mica" for all those minerals that are hydrated micas, and the authors will follow this suggestion.

Mica in the unweathered state has a basal "d" spacing of 10 A, whereas the weathered, or hydrous, mica has a basal spacing that is directly dependent upon the saturating cation. In short, differential cation treatment of mica minerals will reveal the degree of weathering. Unweathered mica is tightly bonded by the interlayer cations and will maintain its 10 A basal spacing regardless of treatment. Weathering, however, with its accompanying cation depletion, results in a progressively greater response to differential cation treatment.

With respect to a monogenetic soil profile, the hydration effects of such divalent cations as Ca^{++} and Mg^{++} will be greatest in the A horizon and will gradually diminish toward the parent material. This is in accord with the concept that hydrous micas will dominate the surface horizon, whereas the D horizon will be composed of the unweathered, unhydrated form.

An example of such a monogenetic profile of weathering was first found in the Shackham Brook watershed located near Cortland, N. Y. The clays were saturated with 1 *N* MgAc (50 ml to 150 mg clay) and

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