In an effort to gain information as to the source of the green color the following procedure was carried out:

To 2 ml. of water containing 5 mg. of penicillin (Brand II) was added 5 ml. of 95-per cent ethyl alcohol, 3 ml. of absolute ether, 1 ml. of diazotized *p*-nitroaniline reagent, and 3 ml. of 5-per cent sodium carbonate. The resulting color was of the same olive green as was seen in the above determinations. Two drops of concentrated hydrochloric acid, when added to this mixture, caused the olive green to fade and a brownish-yellow to appear. Upon realkalinizing, the olive green reappeared. It was also found in the above procedure that the color (olive green) formation required the presence of the 95-per cent ethyl alcohol, but did not require the absolute ether. A water solution of penicillin (Brand II), treated without the alcohol, gave a yellow-orange color upon diazotization.

The foregoing procedure was carried out on several other standard commercial penicillin preparations used clinically. In all cases the results were the same. However, a sample of pure crystalline penicillin sodium G and of "pure" penicillin sodium[1] did not give the slightest adverse chromogenic reaction, but did give a yellowish color with the diazotized *p*-nitroaniline reagent.

It was observed that a few of the yellow crystals (pure penicillin is colorless) of the standard preparations, when shaken with absolute ether, failed to dissolve. However, when a 35-ml. water solution of penicillin (Brand II) plus 1 ml. of 10 N sulfuric acid was extracted for 2 hours in the usual manner as for the determination of phenols, the ether extract, when treated with the diazo reagent, alcoholized, and then alkalinized, gave the olive green color. The ether extract itself had a yellow tinge, while the extracted water solution, which had been pale yellow, had lost its color.

From the evidence presented we may conclude that (a) those commercial preparations of penicillin tested contain some substance (or substances) which gives an adverse chromogenic reaction in the alcoholized, alkalinized, diazotized medium used in determining free phenols according to Schmidt; (b) it is probable that the yellow color in the penicillin preparations is responsible; and (c) pure penicillin G (sodium salt), when alcoholized, alkalinized, and diazotized, does not give the adverse chromogenic reaction, but does give a yellow to orange diazo reaction.

Reference

1. SCHMIDT, E. G. *J. biol. Chem.*, **150**, 69–73.

[1] Kindly supplied by Merck & Company, Inc., Rahway, New Jersey.

A Method of Growing Dense Cultures of *Paramecium*

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The method described below will produce very dense cultures of *Paramecium* substantially free from other *Protozoa* other than minute forms which do not interfere with the use of the material for class purposes. Variations in the method have been tried, but none has given as good results.

Obtain a glass jar about 7 in. deep by a 4- or 5-in. internal diameter. Place in this a tripod made of glass rod or tubing, with its top about 1½ in. below the top of the jar. The tripod consists simply of a ring of tubing a little smaller than the jar, with three legs fused to it.

Pick off the grass blades from some hay, lay them parallel, and cut to length, so as to make a mattress of them, resting on the glass tripod. The mattress should be about ¾ in. thick when pressed down a little.

Fill the jar with tap water to such a depth that the grass mattress is just awash at the top, and then place the jar against the side of an embedding oven set to about 50° C. (The jar is outside the oven, in the room.) With normal room temperatures, this should result in a temperature gradient across the jar, varying from about 25° C. near the oven to about 23° C. away from the oven. Place a lid on the jar and leave for a few days until a thin, whitish scum commences to form on top. Inoculate with *Paramecium*, and leave for another 5 or 10 days, examining from time to time to see that the grass keeps just at or below the water surface. Add a little distilled water if necessary. At the end of this time, a heavy, whitish pellicle should have formed, with enormous numbers of *Paramecium* hiding just at the junction of pellicle and jar at some point at which the temperature suits them. Best results are obtained when the cool side of the jar faces the window, but is shaded from sunlight.

After a week or two, "lagoons" or clear spaces about ¼ in. in diameter will appear in the pellicle. If they do not do so, they should be made by cutting the pellicle from the glass all round. These lagoons are ringed with a whitish "fuzz" consisting of vast numbers of *Paramecium* in almost pure culture. The water below the tripod will be murky with bacteria and minute ciliates, which would seem to provide a reservoir of food for the *Paramecium*.

The culture will remain rich for some weeks and

then fall off rapidly. If a new one is started every three weeks, a continuous supply of *Paramecium* for classes of many hundreds can be maintained.

For success it has seemed essential to (1) keep the grass mattress just awash and the grass about ¼ in. from the jar where the *Paramecium* grow best; (2) maintain a temperature gradient roughly between 23° and 25° C.; (3) use tap water free from injurious metals to start, and distilled water for later additions; (4) keep the jar covered with a loose lid; (5) try to encourage lagoon formation; (6) keep the grass blades at right angles to the temperature gradient, which should be in the direction of the incident light; and (7) shade from sunlight or really bright daylight.

Letters to the Editor

Convulsive Effects of Antibiotic Agents on the Cerebral Cortex

The lack of clinical evidence of neural toxicity when penicillin is administered systemically or intrathecally has led to the assumption that penicillin has no adverse effect upon the physiologic processes of the nervous system. The singular dearth of experimental studies of the effects of antibiotic substances applied to the brain has perpetuated this erroneous impression.

Both clinical and experimental studies on animals and man indicate that penicillin may produce convulsive manifestations when applied to the cerebrum. During systemic administration for conditions other than primary ones of the central nervous system, the electroencephalogram was found to be abnormal in more than 60 per cent of a series of 51 cases. Control records before and after penicillin therapy usually showed norm l tracings. Large doses of penicillin injected intrathecally in man (40,000 units) or monkey (10,000 units) may give rise to generalized convulsions followed in some cases by coma and death. The application of as little as 250 units of penicillin to the cerebral cortex of the macaque may induce epileptic attacks. These convulsive phenomena are not due to impurities in the preparation, for they have occurred with penicillin made by ten different manufacturers and with purified crystalline penicillin.

Streptomycin applied to the cerebral cortices of cats and monkeys in doses of 1,250 units induced convulsive manifestations in 30 per cent of the cases. Electroencephalographic records at such times showed slow waves and spikes with subsequent decrease of cortical activity lasting for one to three hours. Cisternal injection of 2,500 units of streptomycin in the monkey induced signs of severe cerebellar dysfunction.

Streptothricin applied to the parietal cerebral cortex in doses of 5,000 to 10,000 units produced clinical and electroencephalographic convulsive manifestations. Although these phenomena usually disappeared spontaneously in two to three hours, in two monkeys they persisted for two weeks. At necropsy the brains of these animals showed extensive softenings with perivascular petechial hemorrhages.

Actinomycin injected into the cerebral cortex or cisterna magna in a dose of 1 mg., after a latent period of nine hours produced severe prostration, fasciculations, and convulsions with death in one to seven days. At the site of injection into the cerebral cortex a severe necrobiotic reaction with edema and petechial hemorrhages was found.

Clavacin, when injected into the cerebral cortex in doses of 5 to 10 mg., induced clinical and electroencephalographic manifestations of convulsive phenomena with a marked decrease in spontaneous electrical activity of the brain.

There appears to be a relatively wide margin of safety between the antibiotic concentration and convulsive threshold of penicillin and streptomycin. Such does not appear to be the case for streptothricin, clavacin, or actinomycin.

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The Genus *Aspergillus*

In his review of Thom and Raper's *Manual of the Aspergilli* (*Science*, 1945, 102, 460–461) Dr. B. O. Dodge says: "It is gratifying to see that the authors have continued to maintain that the generic name *Aspergillus* should apply not only to the conidial stage but also to the ascosporic stage. The genus *Aspergillus* is here to stay, regardless of rules of nomenclature." This statement should not be allowed to pass unchallenged by those who believe that progress in any branch of science dealing with living organisms will be facilitated by precise designation of the organisms concerned, and that such precision can best be attained by conforming to an established procedure based upon conference and agreement between as many as possible of those who are interested in such matters. In the mycological field, such a procedure is set forth in the International Rules of Botanical Nomenclature. Admittedly imperfect and incomplete (what codification of practice in other fields is perfect and complete?), the rules in their present form represent an orderly development of careful and intelligent thinking on the subject of nomenclature and are entitled to the serious consideration of all who use names subject to these rules. In the very rare cases in which it seems impossible