ELEMENTARY BACTERIOLOGICAL STUDIES.

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No one can doubt the importance of the study of bacteria in courses in botany and hygiene, but the difficulties of technique and the time required for preparation have prevented such studies from being generally undertaken in secondary schools. The usual methods of making, neutralizing and sterilizing gelatin and agar media require too much time and experience. This has brought about a wide use of the potato as a culture medium, but no opaque medium can compare with a transparent one for this work. The potato also often resembles the bacterial colonies in color and no separation of colonies can be made in a solid medium.

Of course the ideal medium would be one which is faintly alkaline, nutritive, sterile, transparent, and capable of being liquefied and used without much preparation. Searching for better simple media I have tried to devise some methods of using the raw white of egg, some fruits, gum tragacanth or starch compositions but without success. The following simple methods, however, have given good results. The time stated as required is approximate.

A. Potato. Cut potatoes in half, boil ten or fifteen minutes, immerse while hot in a hot solution of aniline blue. The boiling may be done in the aniline solution. Transfer with sterile fork to sterile jelly glasses, containing a little sterilized water. Time required, twelve to eighteen minutes. The colonies, formed after inoculation, generally remain uncolored and a pellicle may be found and studied on the water in the glass. Gas bubbles may often be seen.

Aniline black is ordinarily hard to obtain at drug stores but a commercial black dye, presumably aniline, proved satisfactory also in coloring the potato. A strong litmus solution or paste gave good results and those obtained with black ink were fairly good. The litmus has the advantage of indicating acidity but it is more difficult to color the potato evenly with it. Uncolored carrots are suitable for this work. Beets and potatoes stained with aniline red or red ink are less satisfactory, staining the bacterial growths red. Sweet potatoes and parsnips, unstained, gave only fairly good results, their color not contrasting so well with those of the colonies. The colonies show more plainly on ordinary uncolored potatoes if the potatoes have been boiled with a little gelatin.

B. Beef broth. 1. Dissolve 2½ grams of Liebig's extract of beef in 1000 c. c. of boiling water. Time required, 5 minutes.

2. Boil one pound of lean chopped beef in a little water for from thirty to sixty minutes. Filter and dilute to one litre. Time required, forty to ninety minutes.

Place a part of the broth obtained by either of the above methods in test tubes, each about one fourth full, plug with cotton, place tubes erect in water a little deeper than the medium in the tubes, cover the vessel and boil for twenty minutes on three successive days.

C. Gelatin. To 500 c. c. of broth (above) add 60 grams of sheet gelatin and raise the temperature slowly. Do not boil. When dissolved add a solution of sodium carbonate, carefully, till faintly alkaline to litmus. Time required, ten to twenty minutes. Pour into tubes, sterilize on two successive days for twenty minutes. Do not heat more than is necessary. Place the tubes in a slanting position while cooling after sterilization.

D. Agar. To 500 c. c. of broth (above) add 10 grams of agar. Boil till dissolved, add sodium carbonate solution *slowly* till faintly alkaline and pour into tubes. Time required, twenty to forty minutes. Sterilize by boiling for fifteen minutes on each of three successive days.

Media should be made in the presence of the pupils if possible.

Suggested Exercise. Time, two to four hours. 1. Preparation of colored potato media or carrots. Inoculate from water, milk and dust, respectively, and set aside. Also set aside a few sterile dishes of media for controls.

2. Preparation of beef broth tubes. Inoculate as before and add a drop of formalin to some of the tubes.

3. Preparation of agar or gelatin tubes. When liquid and not too warm inoculate and pour contents into sterile Petri dishes. Allow some tubes to harden in a slanting position and inoculate. Put one or two on ice.

4. When colonies develop describe their size, shape, color, number; note the odor of the cultures. Compare the controls. Solid media should be examined for evidence of gases, liquid media for any pellicle formed on the surface or any sediment or cloudiness; gelatin for any trace of liquefaction. Note changes from day to day.

5. Describe live bacteria under high power (if possible use one-twelfth objective). Use stained slides showing different forms and make a classification based on form.

6. Discuss occurrence, variation in size, form, motility, color, and appearance; classification, identification, food, economic relations, use of antiseptics and sterilization based on above studies.

The following topics are suggested for library, essay work and discussion:

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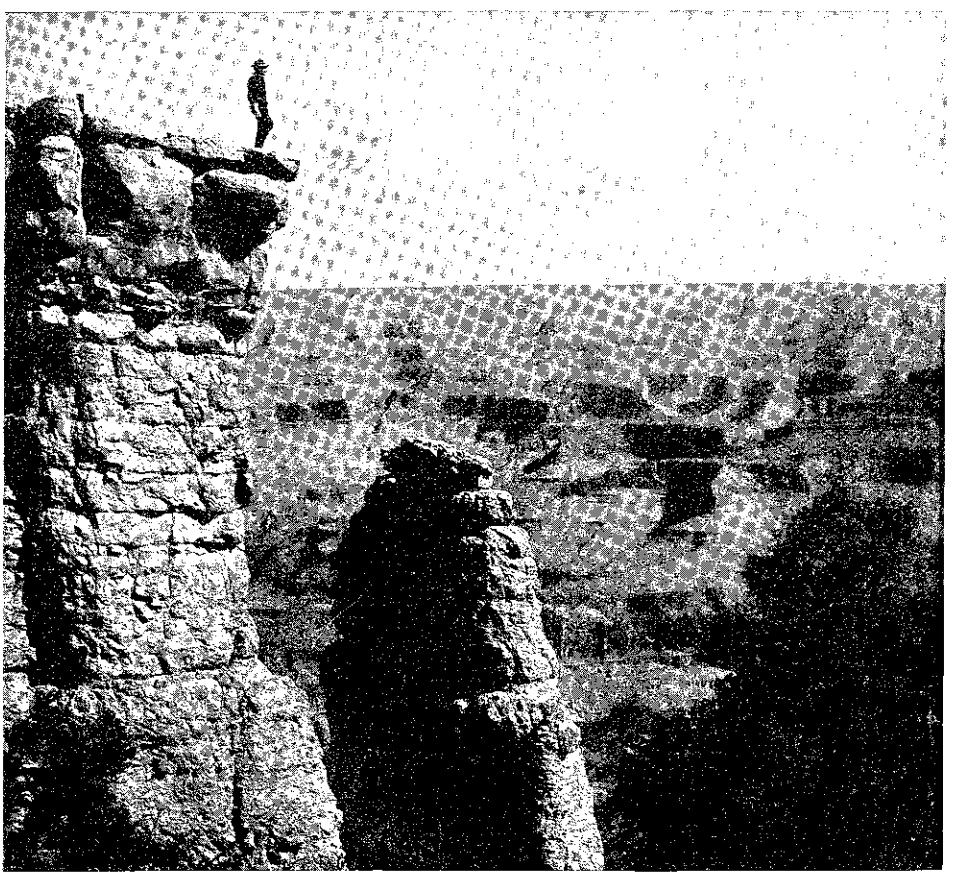
1. Tuberculosis.
2. Typhoid fever.
3. Smallpox and vaccination.
4. Diphtheria and its antitoxin.
5. Pneumonia and influenza.
6. Public health, officers, methods and regulations.
7. Fermentation, sterilization, antiseptics, and preservatives.
8. Nitrification.
9. Some bacterial diseases of plants.



Scene in Grand Canyon, Santa Fe Railroad