

ter for laboratory work and clearing house of zoological knowledge generally, with bearings on animal and human interests in all parts of the world. But such has the Zoo grown to be, and, in view of the shrinkage of space and the development of countries unopened a century ago, its importance can hardly be overestimated.

If its contributions to science are great, so also are its interests and responsibilities in other spheres. Its experience and ascertainments have a strong bearing on the conduct of men towards animals. The ethics of vivisection, of teaching animals to perform and of hunting and shooting them indiscriminately, and the provision against their ultimate extermination are all matters of concern to the society, and its opinion on them must carry great weight. Of all possibly none in certain countries is more important than the safeguarding of native fauna from the ruthless pressure of man. It is for this reason partly that photographs, such as those we are now publishing, have become for so many a welcome substitute for the traditional spoils of the hunter. In the last resort the question rests on the state of public opinion, which at home can hardly be fostered better than by a sight of the animals themselves—especially in such surroundings as approximate as nearly as confinement or restrictions will permit to natural conditions. And it is for this reason that the establishment of the Zoo's new park at Whipsnade is so happy an innovation. According to the able secretary of the Zoo, Dr. Chalmers Mitchell, it may be regarded as a link between the Zoo of an older fashion and those still larger reserves which statesmanship has already set apart as sacrosanct for native fauna in Africa, North America and elsewhere. The centenary of the Zoo and the prospect of the opening of the Whipsnade estate next year should give a new orientation to this practical branch of animal-keeping, and serve to enforce more strongly than ever upon a nation with immense zoological responsibilities the nature of its trust.—*The London Times*.

## SCIENTIFIC APPARATUS AND LABORATORY METHODS

### AN APPARATUS FOR THE STUDY OF MAT- FORMING FUNGI IN CULTURE SOLUTIONS

In recent years considerable progress has been made in the physiological study of fungi, particularly as regards their organic nutrition. The technique usually employed in such work has been to grow the fungus in flasks containing a sterile liquid medium, and to deduce the results from the amount of growth of the fungus mats and analysis of the medium after growth.

This, in principle, is the same as the methods used for the study of the inorganic nutrition of higher plants. For certain types of work with higher plants Johnston's<sup>1</sup> constant flow apparatus has proved of great help, and it was while trying to adopt the principle of this for the growing of mat-forming fungi that the apparatus to be described was tested out. While its use was primarily intended for the growing of fungi on liquid media under constant hydrogen-ion concentration, it can be seen that the method readily lends itself to studies regarding the inorganic and organic nutrition of micro-organisms.

There are many objections to be raised against methods now in use for the study of micro-organisms in culture solutions under constant pH. Sideris<sup>2</sup> enumerated some of them, but his own apparatus leaves much to be desired. By means of tubing he withdrew a sample of the medium in the flask in which the fungus was growing, titrated it to the original pH of that flask and then added acid or alkali to the remaining solution in proportion to its volume. Quite apart from the fact that it requires extreme care to adjust the pH in this manner, the important point seems to have been overlooked that the composition of the medium in the flask is being changed after each titration. While the apparatus may have been suitable for the particular work in hand, it does not lend itself to any work in which comparison of the results obtained is to be the basis of the conclusions drawn, for each flask would have contained a medium of different composition from the others.

Obviously the most desirable type of apparatus would be such that it would allow constant flow of a fresh medium of the same composition under the mats during the whole period of their growth. Since this is only practical in certain exceptional cases, the next best step would be to drain at intervals all the medium from the flask and refill from a reservoir. In this way the method adopted approaches as closely as is desired to the method of constant flow. This is the principle of the apparatus in the accompanying diagram.

The flask *A* is a reservoir containing the medium of desired composition and adjusted to any pH at which the fungus is to be grown. The glass tube *F* is connected at *C* to a Y tube, thus allowing two cultures to be grown on the same medium. Any number of these small flasks may be connected up in this manner, this being especially desirable when an average weight

<sup>1</sup> E. S. Johnston. "An Apparatus for Controlling the Flow of Nutrient Solutions in Plant Cultures," *Jour. of Plant Physiology*, 2: 213-15. August, 1927.

<sup>2</sup> C. P. Sideris. "An Apparatus for the Study of Micro-organisms in Culture Solutions under Constant Hydrogen Ion Concentrations," *SCIENCE*, July 4, 1924, vol. 60, No. 1540, pp. 17-19.

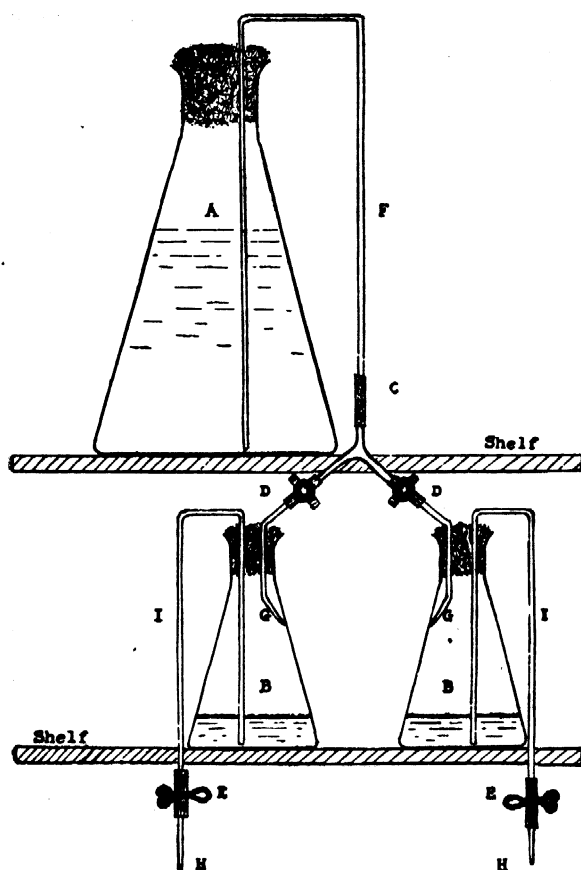


FIG. 1

of mat produced on a given medium is being determined. Flask *B* is supplied with the medium by glass tube *G*, the supply being regulated by screw-clamp *D*. The solution is withdrawn from *B* by means of tube *I* which is joined to the outlet tube *H* by a short piece of rubber tubing having a pinch clamp *E*.

The setting up of the apparatus may be done in several ways, but the following has proved the most desirable. Flask *A* is filled with the medium and tube *E* held in position with cotton-wool. The end of this tube, which is connected by means of a short piece of rubber tubing *C*, is plugged with wool and a paper cap fastened over it. This flask may then be sterilized in the autoclave. The other half of the apparatus is also connected up, the clamps being left loose, as otherwise the rubber tubing is liable to become sealed on the inside. The flasks *B* are left empty and the open ends of tubing plugged up, after which the whole is sterilized in an autoclave. After sterilization flask *A* is placed on an upper shelf with the other flasks below it. Tube *F* is connected at *C* and the clamps fastened in their respective positions.

A vacuum is now connected with one of the outlet tubes *H*, clamp *E* is opened, the fingers are placed

firmly over the cotton of flask *B*, and the clamp *D* is carefully loosened. After a trial it becomes a simple matter to cause the syphon system from flask *A* to work. The clamps are closed when the solution in *B* reaches a desired level, the flasks having been graduated with file marks prior to being connected up. The other clamp *D* is now opened, and so both of the smaller flasks *B* are filled to the proper level. The glass tubes *F*, *G* and *I* are kept full of the solution, air bubbles being removed by tapping during the flow of the solution. Inoculation may now be made by carefully removing the plugs in flasks *B* and introducing the spores or mycelium with a needle.

The solution is withdrawn from *B* by opening clamp *E*, care being taken that when almost all has run out the syphon is not broken. The refilling is now made by opening clamp *D*. The solution should be run down the side of the flask from *G* to prevent the mat being submerged. The above directions are purposely given in full and are meant to serve merely as a guide, for the operation of this apparatus becomes simple after a little experience with it.

A convenient size for flask *A* is two liters, while each flask *B* may be 300 ml. capacity. For most determinations 100 ml. of the medium in each flask *B* has been found to be a suitable volume and is sufficient not to allow it to become exhausted of nutrients in a short period. When the set-up apparatus is not autoclaved prior to its use, the tubes may be sterilized with 80 per cent. alcohol, following by flaming. This method of sterilization has proved very satisfactory. The whole apparatus may be modified according to the wishes of the individual worker, but the principle of an entirely fresh solution being supplied should be retained.

The uses to which this apparatus can be put are not in the least confined to hydrogen-ion control. The solution in flask *B* may be withdrawn as often as desired and determinations made upon it. From experiments already run it seems that an examination of the rate of oxalic acid excretion at different pH values and different sugar concentrations of the original medium would prove to be an interesting study. In addition, a large number of other problems which may be carried out with mat-forming fungi by means of such an apparatus suggest themselves, namely, the secretion of enzymes by the growing organism, the toxic effect and the absorption of certain substances at different stages of growth, and the ability of a well-established fungus mat to increase further in weight on different media supplied from separate reservoirs.

RAIMUND H. MARLOTH

CITRUS EXPERIMENT STATION,  
UNIVERSITY OF CALIFORNIA