The purpose of the experimental forests is to make permanently available, for silvicultural, range, products and other related forest research, areas as fully representative as possible of conditions in important parts of forest regions and large enough to meet present and foreseeable needs. In essence these areas are to be field laboratories for intensive investigative work. A secondary but hardly less important purpose is to provide for the demonstration of results, favorable or otherwise, of widely varying silvicultural and other forest practices. Each experimental forest is to be chosen on the basis that it adequately represents the subregion in which it is located as to forest types and sites and the conditions which underlie types and sites (such as soils, climatic variations and altitudinal range). Wherever possible each experimental forest is to include a "natural area" on which are to be preserved in an unmodified condition examples of the virgin growth of each forest or other vegetative type within each forest region, to the end that the region's characteristic plant and animal life and soil conditions shall continue to be available for scientific and educational purposes.

So far as can now be foreseen, from five to ten experimental forests will be required within each of the twelve or thirteen forest regions specified in the McSweeney-McNary Forest Research Act. Their areas will range from about 1,500 acres to about 5,000 acres, averaging about 3,500 acres, exclusive of the lands to be reserved as natural areas. They will not be so large as to impose any unnecessary burden of administration. Size will be governed primarily by the complexity of the type and by the growth rate of the tree species. The simpler the type and the higher the growth rate the smaller the area that will be required. In a subregion where it is not possible to find a satisfactorily representative single area it may be preferable to establish, as one unit, two or even three separate areas within easy working distance of the same headquarters.

For a natural area 1,000 acres is regarded as the minimum desirable under average conditions, but the acreage will vary with the type of forest involved or, possibly, with climatic and topographic conditions. About a dozen such areas will be required in each forest region. Where areas suitable for experimental forests or natural areas can not be found on existing national forests, consideration will be given to the possibility of acquiring suitable areas by gift or exchange or, as a last resort, by purchase.

Experimental ranges will be established under the same principles as experimental forests.

On the experimental areas scientific and educational uses are to be dominant, commercial utilization and public occupancy subordinate. On natural areas commercial use will be prohibited and public use will be restricted as far as practicable. For convenience of administration and protection the areas will remain essential parts of the national forests on which they are situated, but responsibility for their management and use will rest wholly with the directors of the forest experiment stations. The boundaries of the natural areas and the principles to govern their management are to be established by the forester and are not to be modified except with his approval.

While natural areas will be established primarily to meet the needs of the Forest Service, their use by other research or educational agencies for purposes which do not conflict with Forest Service projects will be allowed under appropriate cooperative agreements approved by the forester.

The readiness with which title to lands can be established under certain of the public-land laws gives rise to some uncertainty at present as to the ability of the Forest Service to safeguard the integrity of the experimental forests and ranges and the natural areas from adverse occupancy and use, but it is believed that as soon as the system has taken definite form and its vital importance to public welfare is established and recognized Congress will make legislative provision for preserving the areas permanently.

The reference in the regulation to primitive areas repeats the language of an earlier regulation under which a comprehensive system of primitive areas is now taking form on the national forest.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

CULTURE MEDIA FOR OPALINIDAE

THERE seem to be three major desiderata in culturing Opalinids: (1) To supply predigested food. Without it I doubt the success of Larson and Allen's[1] experiments or any others. Despite Konsuloff's suggestion[2] that *Opalina* manufactures digestive enzymes and pours them into the "cecal" chamber of the frog host, there to aid in the further digestion of food for

[1] Larson and Allen, "Further Studies on the Reaction of Opalina to Various Laboratory Culture Media," *Univ. Kansas Science Bulletin*, 18: 8, April, 1928.

[2] Konsuloff, "Untersuchungen über Opalina," *Arch. f. Protistenk.*, 44, 3, March, 1922.

the Opalinas, we have no *evidence* of any digestion of any sort by Opalinids. (2) To avoid free oxygen in the culture fluid. (3) To avoid contamination of the culture medium. None of the several culture methods that have been suggested since the time of Pütter's first studies provide the first two desiderata mentioned. The third can perhaps be secured by frequent transfer of the animals to new culture fluid. Supplying predigested food or foods may not prove difficult. On the other hand, to keep the culture free of oxygen is not a simple problem. It requires a technique not yet developed, so far as I know, for culturing any protozoon, except such as will thrive within an agar or gelatin medium. Frequent changing of cultured Opalinids to fresh culture fluid without introduction of considerable oxygen by exposure to the air involves still further technical difficulty. It could doubtless be done with the aid of a gas mask in an oxygen-free room.

Protoopalinae, when kept in Pütter's or Locke's solution, either with or without bits of the rectal wall of the host, show signs of abnormality within a few hours, often within four hours or so. The large, metabolic chromosomes in the huge nuclei of these binucleated Opalinids, when carefully observed by one familiar with their usual appearance, show features which I have interpreted as signs of abnormality. "Sweating" of these chromosomes along their edges begins and develops increasingly, beads of what seems to be chromatin, judging by the staining reactions,[3] appear and increase in size and number. These beads of chromatin sweat on the edges of the macrochromosomes have not been observed in the nuclei of Protoopalinas freshly taken from their host. It seems likely that they arise from an over-emphasis upon a normal process in the metabolic chromatin, making visible the sort of giving off of metabolic products that constantly occurs, only so slowly that they are carried off in liquid form as rapidly as they are developed. In addition to this sweating of the chromosomes, one observes in Protoopalinas kept a day or so outside the host a clumping of the chromatin in ways not noted in freshly taken material. This seems further indication of abnormal condition, as are, also, a slowing of the motion of the cilia and a delaying of the completion of fission, even when well started. These indications of "abnormality" can not be as well observed in the multinucleated Opalinids, whose nuclei, in all species, are much smaller than those in *Protoopalina.*

These facts, and the further fact that in this country Protoopalinas are available for study only in a few regions and in the northeastern states not at all, have made me hesitate to attempt to develop a culture medium and culture methods. Without detailed observation of the minute indications of abnormality in the nuclei, such as are seen in *Protoopalina,* it would be difficult to test adequately the fitness of the culture medium, and without confidence in the suitability of the medium conclusions from experiments with cultured animals are unsafe.

On the other hand, given a suitable culture medium and procedure, the prompt response by *Protoopalina* by visible cytological changes under unfavorable conditions might render *Protoopalina* a peculiarly favorable test animal for studies of protozoan physiology.

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[3] Feulgen's chromatin stain not employed.

RAPID STAINING OF PLANT TISSUES IN THE BUTYL ALCOHOL PROCEDURE

THE time and trouble involved in the preparation of sections, which have been lessened by the use of n-butyl alcohol as described by Zirkle,[1] may be reduced further by saturating the last stage of butyl alcohol with safranin and leaving the objects in it overnight, and by counterstaining the sections with Light Green S. F. in clove oil.

The second change in paraffin will remove all color except from the specimens, which will remain deeply stained and so may be readily oriented for sectioning. The sections on the slides, on being taken from xylene which dissolves the paraffin, should be dipped in a 1:1 mixture of absolute ethyl alcohol and xylene and then counterstained. The slides may be handled separately, in which case a drop of the clove oil solution of light green may be added from a glass rod. This should be flooded within a few minutes with xylene, the slide then redipped in the alcohol-xylene mixture and returned to xylene whence it can be mounted at leisure. If the spiral spring clip and Stender dish described by Chamberlain[2] are used, 15 slides may be handled at one time and should be immersed in the stain.

This method has proved simple, rapid and effective with the various stem tissues tried. With buttercup roots, however, it was found necessary to add a stage of absolute ethyl alcohol just before counterstaining and to leave in this stain half an hour or longer.

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[1] SCIENCE, 71: 103, 1930.
[2] "Methods in Plant Histology," Chicago, 1924.