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.