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Effect of Activated Charcoal in Agar on the Culture of Lower Plants

Proskauer and Berman[1] have described a technique for culturing green organisms such as filamentous algae and moss protonema on an agar substrate containing activated charcoal which may simulate conditions found in nature. They ascribed the resulting morphological changes primarily to a decrease in the amount of light transmitted by the blackened agar, and considered their technique a simulation of natural soil conditions. We have performed similar experiments which also show that moss development is altered on charcoal–agar, but which require another interpretation.

The moss, *Funaria hygrometrica*, was grown on an artificial substrate (modified Knop's solution supplemented with A–Z solution and EDTA complex in 2% agar[2]. The agar was supplemented with various concentrations of activated charcoal, 4% MnO_2 (crystalline limonite) or soil).

On charcoal–agar a series of characteristic alterations in development were observed[2]. The most noteworthy were: a limited development of protonema, acceleration of bud formation and the absence of a spreading caulonema on the agar surface. In addition to the "fairy ring" of gametophores typical for this type of moss, the cultures frequently (with suitable illumination up to 100% of the protonema) developed a single bud in the middle of the protonema. This last phenomenon occurred only by direct culture on agar containing 0.05% or more charcoal. The lower concentrations resulted in only a slight darkening of the medium. This phenomenon

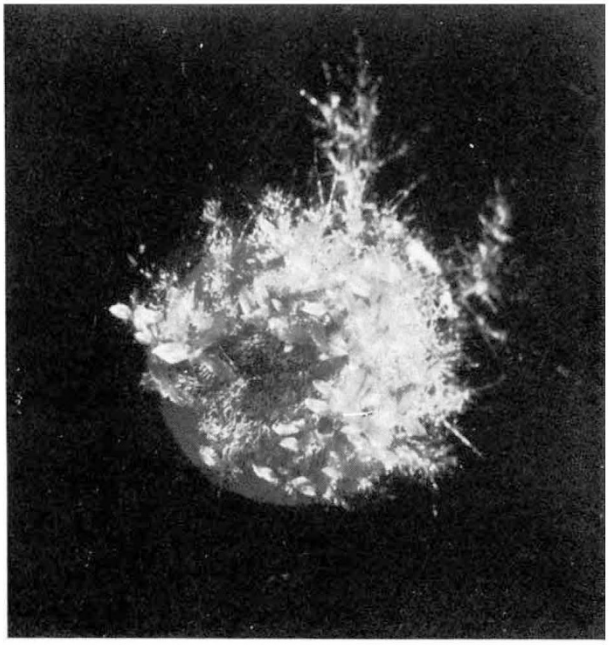

Fig. 1 Protonema from *Funaria hygrometrica* grown on colourless agar surrounded by charcoal-agar. The protonema is inhibited, while bud formation is enhanced by the adsorption through the charcoal. × 5.

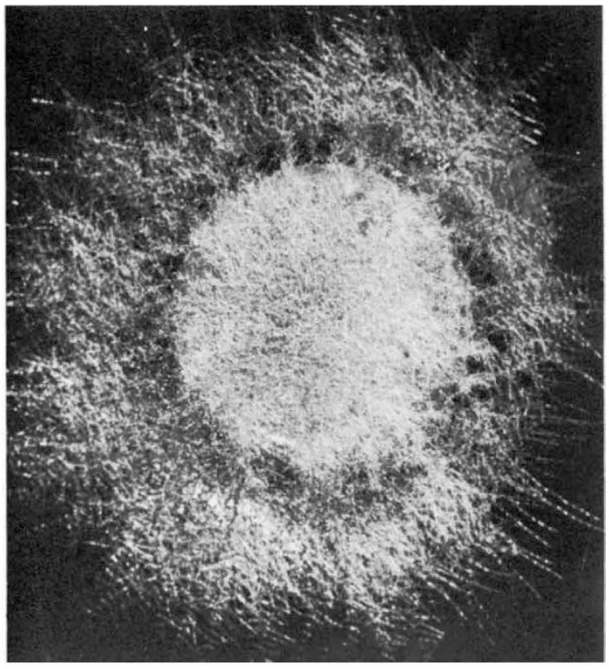

Fig. 2 Protonema of *Funaria hygrometrica* of the same age as in Fig. 1 grown on colourless agar surrounded by MnO_2-agar. Note the spreading of the protonema over the MnO_2-agar; bud formation has not yet started. × 5.

was not observed on limonite or soil–agar, nor did it occur on charcoal–agar covered with colourless cellophane. All these morphogenetic changes were observed when charcoal–agar was covered with a layer of colourless agar up to 4 mm thick. All the changes except central bud formation occurred when the protonema were cultivated on a colourless agar disk as much as 28 mm in diameter which was separated by a glass wall from charcoal–agar in the rest of the Petri dish (Fig. 1).

When limonite was employed, which absorbed nearly as much light as charcoal, bud formation was barely accelerated and the protonema spread out in the same manner as on colourless substrate (Fig. 2). The alterations in development, therefore, cannot be ascribed to light absorption by the agar—which, incidentally, leads also to a change in the relative humidity of the substrate surface. Our results are best interpreted if one assumes that activated charcoal adsorbs substances which influence the development of the protonema *in vitro*. For central bud formation, these substances pass through the substrate; for the other changes, through the gas phase of the Petri dish from where they are also adsorbed. Because it is known that the development of moss protonema is regulated for the most part by substances which are liberated into the substrate by the protonema themselves[3], the adsorption of such substances by activated charcoal is not surprising[4]. Our results do not disprove that in nature, adsorption of these substances onto soil particles may play a role in moss development.

Although our observations support those of Proskauer and Berman[1], who based most of their work on the rhizoid system of mosses, they further indicate that adsorption may be a critical factor, and that charcoal–agar simulates more than just natural light conditions.

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