Isolation and identification of the orange pigment in the fungus *Cladochytrium replicatum*

Among the uniflagellate Phycomycetes, carotenes occur commonly in several species of *Allomyces*, in *Blastocladiella* sp., and in *Karlingia rosea*. The pigments of these fungi have been discussed by HAXO[1]. Although *Cladochytrium replicatum*, a monocentric chytrid, was first described by KARLING[2] as having "a bright golden brown globule" in its zoospores, no identification of the major orange pigment of this fungus has been published to date. A pure culture of *C. replicatum* was sent to M. S. FULLER in 1956 by Dr. L. G. WILLOUGHBY, Freshwater Biological Station, Ambleside, England. According to WILLOUGHBY, this fungus, although orange, rarely produces sporangia when cultured on agar. Our own observations on both Emerson's YpSs agar (Difco) and CRASEMANN's[3] medium 1B plus Difco yeast extract (1 g/l) confirmed the absence of sporangial production under such conditions. However, the plants were still orange-brown in color with the pigment localized in the rhizomycelial swellings. The present study was undertaken to isolate and identify this pigment.

A suspension of rhizomycelium fragments, prepared by grinding the fungus for 30 sec in a Waring Blendor with distilled water, was used to inoculate Petri dishes containing YpSs agar over which a piece of sterile cellophane dialysis membrane had been placed. After 3 weeks growth at 25°, the fungus was harvested by pulling the dialysis membrane from the agar surfaces. Unfortunately, this latter step was made difficult by the partial decomposition of the cellulose by *C. replicatum*.

Acetone was used to extract the pigment from the rhizomycelium. Following extraction, the pigment was transferred to petroleum ether. This was done by mixing petroleum ether, the acetone extract, and 5 % aq. NaCl in a separatory funnel. The hypophasic acetone–NaCl–water layer was removed and the remaining petroleum ether with the pigment was evaporated by means of a water aspirator to a volume of approx. 2 ml. This pigment in petroleum ether was transferred to a chromatographic column containing magnesium oxide–Johns-Manville Celite 545 (1:2, w/w). This column was developed with suction using petroleum ether containing 1 % acetone by volume.

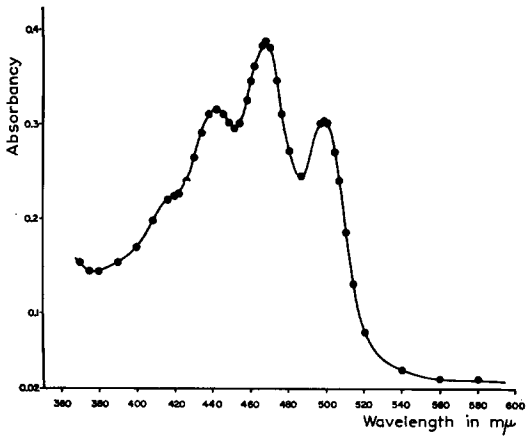

Fig. 1. Absorption spectrum for lycopene extracted from the fungus *Cladochytrium replicatum* and dissolved in petroleum ether.

Following development, only one pigmented red-orange band was present. This band was removed from the column and transferred as above to pure petroleum ether. Identification of the pigment was made by measuring the light absorption at 2-mμ intervals in wavelength from 375–700 mμ with a Beckman DU spectrophotometer. Fig. 1 shows the absorption curve obtained for the pigment isolated from *C. replicatum*. The absorption maxima are at 443, 468, and 498 mμ, and the curve is a characteristic one for the carotene, lycopene. TURIAN AND HAXO[4] published a similar curve with absorption peaks at 445, 470, and 500 mμ for lycopene which they extracted from the gametophytic plants of *Allomyces javanicus*. The authors are indebted to Dr. L. J. WILLOUGHBY for making this isolate of *C. replicatum* available for their study.

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The effect of heart mitochondria on glycolytic systems from brain and heart

In recent years a number of investigators[1,2] have utilized the Pasteur effect in attempts to elucidate regulatory mechanisms involved in carbohydrate metabolism. Among these have been the studies of AISENBERG, REINAFARJE AND POTTER[3], AISENBERG[4] and more recently, CREMER[5]. AISENBERG *et al.* found that liver mitochondria inhibited glycolysis of a brain supernatant system, while CREMER described an inhibitory or stimulatory effect of rat-liver and -brain mitochondria, respectively, on the same system.

CREMER indicated that addition of mitochondria to a glycolytic system prepared from the same tissue would not result in an inhibitory effect in contrast to the studies by AISENBERG *et al.* in which mitochondria were added to a glycolytic system obtained from different tissues. CREMER further suggested that the stimulatory, rather than inhibitory, action of brain mitochondria on glycolysis of brain supernatant was probably due to the presence of a high hexokinase activity in brain mitochondria.

During our studies of oxidative phosphorylation of normal and failed guinea pigs[6], it became apparent that heart mitochondria from both the normal and failed animals produced a stimulation instead of a depression of a glycolytic system obtained from brain tissue.

The present report concerns studies the data of which appear to be pertinent to the above work. It describes experiments in which both normal and "failed" guinea-pig-heart mitochondria were added to either brain-or heart-supernatant glycolytic systems under aerobic conditions. The mitochondria from failed guinea pigs were

Abbreviations: ATP, adenosine triphosphate; DPN, diphosphopyridine nucleotide; EDTA, ethylenediaminetetraacetate.