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Carbon Dioxide-dependent Morphogenesis in *Arthrobotrys conoides*

Carbon dioxide has been shown to influence morphogenesis in micro-organisms, higher plants and animals. Among the processes affected by carbon dioxide (or bicarbonate) concentration are sporangial differentiation in *Blastocladiella emersonii*[1], mould-yeast dimorphism[2,3], spherulation in *Coccidioides immitis*[4], sporulation by bacteria[5] and fungi[6,7], root development by pea[8], and sexual differentiation in *Hydra*[9]. This list can now be extended to include morphogenesis in *Arthrobotrys conoides*. *A. conoides* is a nematode-trapping fungus which captures prey in adhesive networks of hyphal loops. Usually, these organelles of capture are not formed spontaneously by the fungus but develop in response to the presence of prey. Morphogenesis is induced by a metabolic product of nematodes which has been partially purified[10–12] and designated nemin[13]. The tests described here provide evidence that nemin-induced morphogenesis in *A. conoides* is dependent on carbon dioxide.

Petri plates containing maize-meal extract agar were inoculated with *A. conoides* and stored in air at 28° C until the fungus developed as a single colony which covered approximately one-third of the agar surface in each plate. Some of these colonies were treated with nemin (added as an aqueous extract of ascarids) to induce morphological differentiation and then incubated, with untreated controls, for three days at 28° C in desiccators containing the following concentrations of carbon dioxide in air: (*a*) 0·00 per cent (1·0 N KOH placed in bottom of desiccator); (*b*) 0·03 per cent (air); (*c*) 10 per cent (air adjusted by introducing into the desiccator manometrically measured amount of carbon dioxide from a tank). A quantity of 1·0 N H_2SO_4 was placed in desiccators (*b*) and (*c*) to approximate the dehydrating effect of the KOH in desiccator (*a*). The influence of concentration of carbon dioxide on growth was measured as the change in diameter of colonies during storage in the desiccators, and cultures were examined microscopically for morphological change. The results of independent tests performed in our separate laboratories using two strains of *A. conoides* are summarized in Table 1.

A. conoides grew but formed no traps in the absence of carbon dioxide. Growth in air (0·03 per cent carbon dioxide) exceeded that in an atmosphere devoid of carbon dioxide, and, in air, the fungus formed traps in response to treatment with nemin. In no case did traps develop in controls that were not treated with nemin. Although nemin-induced morphogenesis was carbon dioxide-dependent, the influence of concentration of the gas on growth and trap formation varied with the strain of *A. conoides* tested. Growth and the response of strain *NB* to nemin were both greatest in air, and reduced by an absence or an abundance of carbon dioxide. Strain *C* developed best in air, but the morphogenic response of the fungus to nemin was maximum in an atmosphere containing 10 per cent carbon dioxide. Optimum levels have not been established; but growth and nemin-induced morphogenesis in *A. conoides* appear to be independent functions of carbon dioxide concentration.

Table 1. Influence of Concentration of Carbon Dioxide on Growth and Trap Formation by Two Strains of *A. conoides* treated with Nemin

Reaction	*NB* strain CO_2 (%)			*C* strain CO_2 (%)		
	0	0·03	10	0	0·03	10
Growth*	15	20	10	12	20	8
Morphogenesis†	−	+ +	+	−	+ +	+ + +

* Radial expansion of colony (mm).
† Abundance of traps: −, none; +, few; + +, many; and + + +, very many.

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CYTOLOGY

Absence of Late Replication of a Human X-Ring Chromosome

As autoradiographic examination of human and other mammalian chromosomes after incorporation of tritiated thymidine has revealed a characteristic pattern of replication during the late *S* phase for the heteropycnotic *X* chromosome and some autosomes, the lymphocyte culture technique associated with a simplified autoradiographic method may be used for diagnosis and investigations of the sex chromosomes even in smaller laboratories.

In lymphocytes of an intelligent girl, twelve years of age, with typical features of Turner's syndrome and negative sex chromatin in leucocytes and mucosa nuclei—the patient being one of a series of 40 females with chromatin negative Ullrich–Turner syndrome—we found two cell lines with 45 and 46 chromosomes respectively in a ratio of about 40/60. Cells with 46 chromosomes were characterized by a little odd-shaped chromosome which in 41 out of 113 complete mitoses had a ring configuration. Its diameter did not exceed the length of a chromosome in group 21–22. Bipartite (Fig. 1) and quadripartite configurations were like those shown by Lucas[1] and Gropp[2] on ring chromosomes of No. 18.

Autoradiographic examination using Ilford *K*-2 nuclear emulsion instead of stripping film for the exposure of the fixed, spread and orcein stained chromosomes following incubation for 6 h of tritiated thymidine failed to show late labelling of the ring chromosome which has been considered as a deleted *X*. Grains in 113 mitoses have been counted and *X*/45 ratio[3] was determined for several degrees of labelling. In mitoses which contained a few grains, thus marking late *S* phase, we saw clearly labelled