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Dependence of encystment on encystment enhancing activity in *Acanthamoeba*. ROBERT A. AKINS, SUSAN M. GOZS, and THOMAS J. BYERS, Departments of Microbiology and Zoology, The Ohio State University.

Encystment of *Acanthamoeba* can be induced in Keff's Optimal Growth Medium (OGM) by berenil (EER) and other inhibitors. We have shown previously that BER treatment inhibits multiplication and results in release to the medium of an encystment enhancing activity (EEA) that is not present during logarithmic growth (LG). The % cysts reaches half maximum ~40-50 hr after BER addition and is proportional to EEA concentration. We now demonstrate that the BER function is completed within 5-10 hr, whereas the EEA requirement occurs later and continues throughout cyst wall formation. Growth limitation may be the stimulus for EEA production in OGM since EEA also is produced during the transition between LG and postlog growth and in response to a temperature decrease during LG. In contrast, EEA levels are high throughout LG in a chemically defined growth medium (DGM); an observation consistent with previous evidence that EEA does not induce encystment by itself. Encystment in DGM follows replacement of the medium with glucose-free DGM. High levels of EEA are reestablished prior to cyst formation and

the rate of encystment is proportional to EEA concentration. Thus, although EEA does not induce encystment, it does enhance the response in the preceding cases. Two additional cases have been found in which EEA enhancement could not be demonstrated: encystment in Neff's nutrient-free encystment medium; and encystment induced by glucose-free DGM when amebas that had undergone longterm adaptation to growth in DGM were used. In each of these cases, encystment appears to be independent of exogenous EEA. The relationship between dependence and independence can be reversible, however, since DGPI-adapted amebas readily regain EEA dependence upon cultivation for several passages in OGM before retesting. by (Supported NIH 5 R01 AI75526)