Osmotic Concentration of the Contractile Vacuole of *Amoeba proteus* Following UV-Light Irradiation

The contractile vacuole of *Amoeba proteus* exhibits an atypical appearance, an enlargement in size, and altered contraction rate after micrurgical removal of the nucleus or after the *Amoeba* has been irradiated with UV-light[1,2,3]. Since these changes in the contractile vacuole are rapid with respect to other changes in the cell[1,4-6], it suggests that UV-irradiation may affect the osmoregulatory function of the contractile vacuole. In this paper we report on the osmotic concentration of the contractile vacuole following UV-irradiation.

Materials and methods. The methods for culturing and for UV-irradiation at 254 nm of *A. proteus* have been described[7]. The milliosmolal concentration of the contractile vacuole was determined by a modification[8] of the method described by SCHMIDT-NIELSEN and SCHRAUGER[9].

Results and discussion. About 1.0% of a population of unirradiated cells have enlarged contractile vacuoles, whereas after UV-irradiation about 10% have enlarged contractile vacuoles[1,8]. The milliosmols of the enlarged and unenlarged contractile vacuoles in the control cells were not statistically different (Table). The milliosmolal concentrations for the contractile vacuole contents of the control cells were larger than those reported by SCHMIDT-NIELSEN and SCHRAUGER[9]. This may be due to the different culturing conditions and strains of *A. proteus* used in the 2 investigations.

The UV-dosages used decreased the survival of the cells and altered the function of the nucleus[7]. Although UV-irradiation results in a larger % of the cells having enlarged contractile vacuoles, there is no statistical difference between the milliosmolal concentrations of the enlarged and unenlarged contractile vacuoles of the controls and the irradiated cells.

The similarity of the milliosmolal concentrations in the contractile vacuole between the controls and the irradiated cells suggests that the UV-light may not be affecting the mechanism(s) by which the contractile vacuole contents are regulated. However, there could be an altered ratio of ions, or non-electrolytes, which would not be detected by this method. In summary, UV-irradiation does not result in a detectable altered milliosmolal concentration of the contractile vacuole.

Zusammenfassung. Nach UV-Bestrahlung erweist sich die osmotische Konzentration der pulsatilen Vakuolen von *Amoeba proteus* als unverändert.

Milliosmolal concentration of the contents of enlarged and unenlarged contractile vacuoles of *Amoeba proteus*

	Unenlarged vacuoles	Enlarged vacuoles
Control	68 (30[a]) (30.33[b])	56 (12[a]) (18.61[b])
1000 ergs/mm²	76 (15) (28.56)	80 (15) (29.02)
2500 ergs/mm²	77 (40) (40.62)	49 (15) (16.90)

[a] = No. of samples. [b] = standard deviation of the mean.

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The Production of Stable Steady-States in Mouse Ascites Mast Cell Cultures Maintained in the Chemostat[1]

Sustained growth of randomly phased (asynchronous) fluid-suspension cultures of unicellular micro-organisms in a constant chemical environment, at a constant cell concentration and at a dictated exponential rate, has been achieved in the chemostat[2-9]. Similar attempts with animal cells have been only partially successful[10], limiting the usefulness of the chemostat for the biochemical, physiological and genetical analysis of animal cells in vitro. In this article we summarize chemostat experiments with neoplastic mouse mast cell cultures in which true stable steady-states were established.

The chemostat[3,10] is a device for growing cell cultures in steady-state[11] at preset doubling rates which are smaller than the maximum specific growth rate and which are controlled by one or more (limiting) nutrient factor(s). In essence the chemostat is an agitated culture vessel with an overflow setting the culture volume. Fresh nutrient liquid enters the culture vessel at a constant rate (dilution rate) and cell suspension, including supernatant medium, leaves the culture vessel, under ideal conditions of operation, at the same rate (specific washout rate). In the experiments reported here 2 types of automated mammalian-cell chemostats were used, models I and II, differing only in the design of the culture vessel and the stirring mechanism. Model I, which is an adaptation of the cytogenerator[12] to the principle of the chemostat, consists of a 2-arm culture vessel with an overflow on one arm and an inlet port for liquid nutrient on the other arm. Premixed gases (air, CO_2) under moderate pressures are alternately pulsed to the 2 arms of the growth chamber, accomplishing both agitation of the cell suspension and equilibration of the liquid phase with O_2 and CO_2 (pH control). Model II consists of a round reaction flask-type culture vessel with an overflow, a suspended inlet tube for the afferent nutrient stream which is broken by a dropping device, and a Vibro-Mixer,