

Cell Volume Determinations of *Dictyostelium discoideum*

JOHN BARRAVECCHIO, PAUL BAUMANN, AND BARBARA WRIGHT

Department of Developmental Biology, Retina Foundation, Boston, Massachusetts 02114

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The intercellular water present in pellets of centrifuged cell suspensions of the slime mold *Dictyostelium discoideum* was measured at four stages of differentiation.

Among the many factors which can affect the rate of an enzymatic reaction during differentiation of the slime mold *Dictyostelium discoideum* are concentrations of substrates, products, and effectors. Detailed kinetic properties of an enzyme, its concentration, and the cellular levels of various reactants are of importance in assessing a reaction rate in vivo (4, 5). In past experiments, metabolite concentrations were determined in cell suspensions, samples of which were centrifuged, and the pellet volume was measured. The pellet volume was used as the basis for calculating the cellular concentration of the metabolite in question. No attempt was made to correct for the intercellular water present in the pellet. This note is an attempt to introduce such a correction by determining the intercellular water present in such pellets at different stages of differentiation. ^{14}C -inulin was used as the substance which would not permeate the cells.

D. discoideum was grown and harvested at various stages of differentiation in Bonner's salt solution (1) as described previously (3). Harvesting and all subsequent operations were performed at 4 C. ^{14}C -inulin (1.5 mc/ml) was obtained from New England Nuclear Corp. Radioactive samples were mixed with a scintillation gel (2) and counted in a Beckman scintillation counter model LS200.

A typical experiment was carried out as follows. To 1.5 ml of the harvested cell suspension, 20 μl of ^{14}C -inulin was added. After careful agitation to insure proper mixing, 0.1 ml of the suspension was removed for counting. A 1-ml amount of the remaining cell suspension was transferred to a graduated test tube and centrifuged at $1,000 \times g$ for 15 min. The pellet volume was noted, and 0.1 ml of the supernatant liquid was removed for counting. The volume of cells present in 1 ml of the cell suspension is equal to 1 - 1 (counts before centrifugation/counts after centrifugation). The obtained value ($\times 100$)

divided by the pellet volume gives the percentage of the pellet volume consisting of cells. The experiments were so designed that the pellet volume was always 40 to 60% of the cell suspension. No significant change in the pellet volume was observed after an additional 15 min of centrifugation. Table 1 gives the results of a number of such experiments performed at four stages of differentiation.

As would be expected, the percentage of the pellet volume consisting of cells decreases from

TABLE 1. Percentage of the pellet volume consisting of cells^a

Aggregation	Slug	Culmination	Sorocarp
89	79	60	41
78	73	73	32
85	84	65	47
85	64	69	27
	72		28
	82		31
			46
			24

^a Mean values \pm standard error were: aggregation, 84.2 ± 4.6 ; slug, 75.6 ± 7.4 ; culmination, 66.7 ± 5.5 ; sorocarp, 34.5 ± 8.9 .

the aggregation stage to the sorocarp stage of differentiation. The low values at the sorocarp stage are probably the result of the presence of stalks which prevent the formation of a well-packed pellet. The obtained values are averages of all of the cell types present.

Since the added radioactivity was readily diluted, as expected, by washing, it appears that ^{14}C -inulin is neither bound to nor permeates into the cells. If such binding had occurred, the calculated value for the percentage of the pellet volume consisting of cells would have decreased.

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