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Extracellular Concentrations of Adenosine 3′:5′-Cyclic Monophosphate during Axenic Growth of Myxamoebae of the Cellular Slime Mould *Dictyostelium discoideum*

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On removal or exhaustion of food the myxamoebae of the cellular slime mould *Dictyostelium discoideum* cease dividing and, when deposited on a solid substratum, undergo a characteristic morphogenesis (Bonner, 1967). Starvation thus seems to be the prerequisite, if not the trigger, for the onset of cell differentiation. B. D. Hames & J. M. Ashworth (unpublished work) have shown that 'starvation' cannot be defined, in this instance, as a lack of available nitrogen, carbon or energy sources. Nevertheless there are extensive biochemical changes during the cessation of true exponential growth and the onset of stationary phase (Ashworth & Quance, 1972). We now report a similarly timed change in the extracellular concentration of cyclic AMP (adenosine 3′:5′-cyclic monophosphate) and suggest that this change be used as a biochemical correlate of the imprecise term 'starvation'.

Myxamoebae of *D. discoideum* strain Ax-2 were grown axenically in 700ml portions of liquid medium, in the presence of 86mM-glucose, unless otherwise stated (Watts & Ashworth, 1970). Samples were

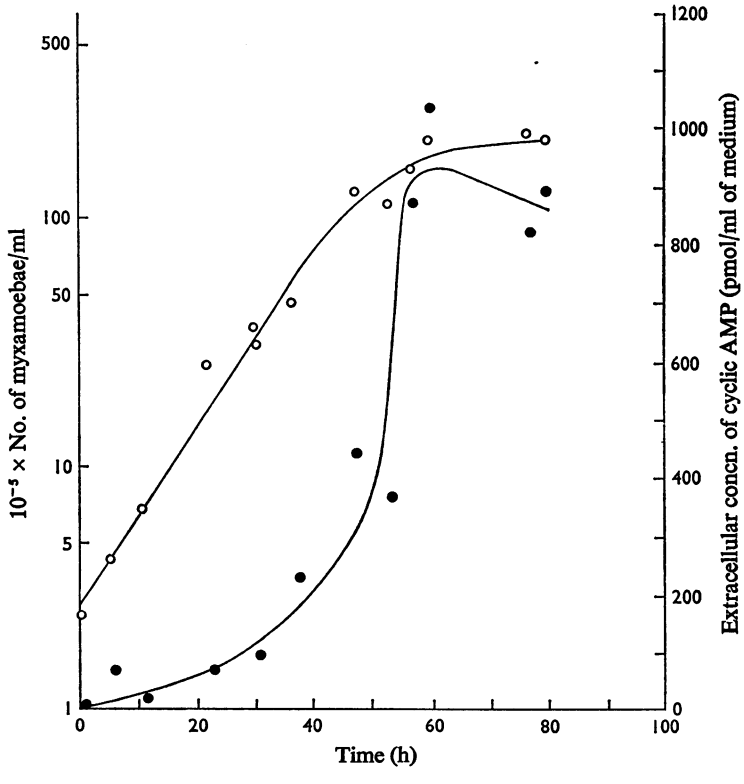

Fig. 1. *Extracellular concentrations of cyclic AMP during the growth of D. discoideum Ax-2*

Cultures (700ml) were shaken at 22°C and the increases in cell number were determined by haemocytometer counts. Samples taken at each time-interval were centrifuged and the concentrations of cyclic AMP in the supernatant were determined (Gilman, 1970). ○, Cell density; ●, concentration of cyclic AMP. The results of two independent experiments have been combined to produce this figure.

Table 1. *Extracellular concentration of cyclic AMP at stationary phase in D. discoideum Ax-2 as a function of glucose-induced changes in cell yield*

Cultures of myxamoebae (60ml) were shaken at 22°C in media containing the glucose concentrations indicated. After several generations, inocula were added to media containing the same concentration of glucose. Cell counts were taken with a haemocytometer throughout growth. Stationary-phase samples were centrifuged and the concentrations of cyclic AMP in the supernatants were determined (Gilman, 1970).

Concn. of glucose in growth medium (mM)	Cell yield (no. of myxamoebae)	Maximum extracellular concn. of cyclic AMP at stationary phase (pmol/ml of medium)
0	1.1×10^7	800
50	1.4×10^7	1350
100	2.1×10^7	1500
150	1.8×10^7	1000
200	1.5×10^7	700
250	1.0×10^7	590

removed at various times after inoculation and the cell density was determined with a haemocytometer. The cells were then pelleted by centrifugation and the supernatants stored frozen. Concentrations of cyclic AMP were determined by the method of Gilman (1970), modified by increasing the sodium acetate buffer concentration from 50 to 250mM. This is an isotope-dilution assay, and involved measuring the ^{3}H-labelled cyclic AMP bound to a purified muscle protein kinase kindly given to us by Dr. M. K. Essenberg. That the material assayed was indeed cyclic AMP and not a non-specific inhibitor of binding was shown by the destruction of the binding-competition activity with ox muscle cyclic AMP phosphodiesterese [obtained from the Sigma (London) Chemical Co. Ltd., London S.W.6, U.K.].

The results are shown in Fig. 1. On the onset of stationary phase there is a sudden rise in the extracellular concentration of cyclic AMP from about 75 to over 1000pmol/ml.

A similarly timed sharp rise in the extracellular concentration of cyclic AMP has been reported to occur with *Escherichia coli* B (Peterkovsky & Gaydor, 1971) and correlated with the exhaustion of glucose in a minimal salts medium. Table 1 shows that this correlation does not apply to *D. discoideum*. Cells were grown for several generations in media containing different concentrations of glucose, which affects cell yield (Watts & Ashworth, 1970). These pre-adapted cells were then used as inocula for 700ml cultures. In all cases there was an abrupt increase in the concentration of cyclic AMP at the end of the exponential phase, resulting in over a tenfold increase in the concentration of extracellular cyclic AMP by the time the stationary phase was reached. The maximum concentration of cyclic AMP reached at stationary phase reflected the cell yield, but was not proportional to glucose concentration. That this increase is not due to lysis of cells containing a higher intracellular concentration of cyclic AMP than is present extracellularly is indicated by the fact that assays of samples obtained by boiling the total culture medium gave the same values as those of the supernatant alone.

Bonner *et al.* (1969) have shown that extracellular cyclic AMP is a chemotactic mediator of the aggregation process during differentiation of the slime mould. The increased concentration of extracellular cyclic AMP on the cessation of growth reported here may thus reflect the transition from the growth phase to the cell-differentiation phase. It is known (Ashworth & Quance, 1972) that a number of enzymes implicated in the differentiation process are also excreted at this time, and this agrees with this interpretation.

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