LEVELS OF CYCLIC AMP IN MURINE L5178Y LYMPHOBLASTS GROWN IN DIFFERENT CONCENTRATIONS OF SERUM

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Murine L5178Y lymphoblasts were grown in different concentrations of horse and fetal calf serum. Intracellular levels of cyclic AMP, determined throughout the growth period of these cells, did not vary significantly. Furthermore, logarithmically growing cells that became stationary did not show the pronounced increase in levels of cyclic AMP that is characteristic of other cell types when they become confluent or quiescent. These results demonstrate that the unrestricted growth of lymphoblasts is independent of the serum concentration used and may be due to an aberration in the cyclic AMP system.

Adenosine 3',5'-cyclic monophosphate (cyclic AMP) has been implicated in the regulation of cell growth (1-8). In fibroblasts the density-dependent cessation of growth is accompanied by a corresponding increase in cyclic AMP levels, which suggests that high levels of intracellular cyclic AMP are inhibitory, whereas low levels permit growth (1, 2, 6, 7). In addition, several neoplastic cell lines or virus transformed cell lines have been shown to be more sensitive to exogenously supplied cyclic AMP or its dibutyryl derivative (dibutyryl cyclic AMP) than normal ones (7, 9-13). Since these "abnormal" cells generally have lower intracellular levels of cyclic AMP than their cells of origin, one distinct possibility is that the cyclic AMP system in neoplastic cells is aberrant (7).

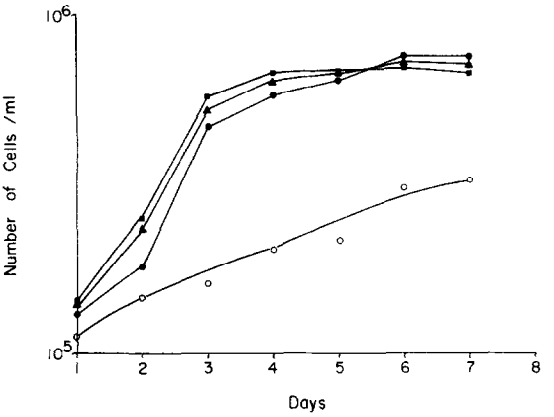
Recently, Seifert and Paul (8) determined the levels of cyclic AMP in sparse and dense cultures of growing and quiescent 3T3 cells. When logarithmically growing cells became quiescent, the levels of cyclic AMP increased. Furthermore, by growing cells in 1% or 10% serum, they demonstrated that the increase in cyclic AMP

was related to the state of confluency and was independent of the contact inhibition of the cells. Thus, cell growth was dependent upon the amount of serum that was supplied to the medium. Seifert and Paul (8) concluded that serum factors are involved in the regulation of cyclic AMP levels.

In order to examine further the influence of serum and cell to cell interaction upon the relationship between cyclic AMP and cell growth, we have determined cyclic AMP levels in L5178Y lymphoblasts in low, medium, and high serum concentrations. These lymphoblasts grow in suspension culture and therefore are not subject to the same type of cell to cell interaction that is characteristic of fibroblasts.

Materials and Methods. Murine L5178Y lymphoblasts (14) were grown in Fischer's medium (Gibco) in an incubator at 37°C containing a gas mixture of 5% CO_2 and 95% air. The cells were washed and resuspended in fresh Fischer's medium supplemented with the appropriate concentration (% V/V) of horse or fetal calf serum (Gibco) for each experimental procedure. The suspension cultures were propagated in 500 ml bottles (Gibco) and aliquots of cells were removed for cell counts and cyclic AMP assays at 24 hour intervals. Cell numbers were determined with a Coulter counter (Coulter Electronics) and a hemocytometer. Viable cell counts were performed according to the procedure of Phillips and Terryberry (15).

Samples for the cyclic AMP assay were obtained from the cultures each day, cooled rapidly to 4°C and centrifuged at 1000 x g for 5 minutes. The medium was then removed and replaced with 1 ml (4°C) of 5% trichloroacetic acid (TCA). The samples were then sonicated briefly (15 to 20 seconds) and centrifuged (4°C) at 2500 x g for 10 minutes. The supernatant fraction was acidified with HCl and then extracted 5 times with 2 ml of ether and lyophilized. Cyclic AMP content was then determined according to the procedure described by Gilman (16), which included phosphodiesterase treatment of duplicate samples to determine the specificity of the assay.



Growth of L5178Y Cells Under Different Culture Conditions

Cells were propagated in suspension culture with Fischer's medium for leukemic cells of mice supplemented with either horse serum or fetal calf serum. The medium contained either 1% (●—●), 5% (▲—▲), or 10% (■—■) serum or was left unsupplemented (○—○). Cell counts were made every 24 hours with a Coulter counter. Cells used for these experiments were washed with Fischer's medium and resuspended in Fischer's medium without serum, and then serum was added to the individual culture to the desired concentration.

Results and Discussion. L5178Y cells were cultured in medium containing 1%, 5%, or 10% horse serum or fetal calf serum (Fig. 1). When the cells were grown in these different concentrations of serum, cell viability decreased between 24 and 48 hours, but the final cell density at days 5 to 7 was not significantly different. When no serum was added to the medium, cell numbers increased only slightly. This indicates that these lymphoblasts require one or more serum factors for growth.

As shown in Table 1, the cells were analyzed at 24 hour intervals to determine intracellular levels of cyclic AMP throughout the experimental period. The levels of cyclic AMP did not differ significantly at any stage of growth. Thus, in medium without serum, the cyclic AMP levels remained approximately 2.56 ± 0.26 pmoles/10^6 cells. Those cultures with serum also did not show the two-fold increase in levels of cyclic AMP that was found in other systems (1-8) when the cells reached confluency or became quiescent. Furthermore, additions of fresh serum to the medium after 7

Cyclic AMP Levels in L5178Y Lymphoblasts

Serum Concentration (%)	Growth Phase	pmoles cAMP/10^6 cells	
		Horse Serum	Fetal Calf Serum
0	Stationary	2.56 ± 0.26	2.56 ± 0.26
1	Logarithmic	2.14 ± 0.94	1.74 ± 0.46
1	Stationary	2.13 ± 0.83	1.28 ± 0.31
1		(2.12 ± 0.78)	(1.53 ± 0.57)
5	Logarithmic	2.22 ± 0.41	1.44 ± 0.47
5	Stationary	2.31 ± 0.52	1.02 ± 0.12
5		(2.10 ± 0.48)	(1.16 ± 0.59)
10	Logarithmic	1.21 ± 0.22	1.67 ± 0.81
10	Stationary	1.37 ± 0.18	1.53 ± 0.37
10		(1.15 ± 0.25)	(1.05 ± 0.71)

Levels of cyclic AMP were determined each day during the experimental period, and the results are expressed as the mean values ± the standard error of the mean. The values shown represent at least three assays/experiment for each time period tested. Values in ( ) are the means ± the standard error of the mean for all cyclic AMP determinations made during the course of the experiments. These values represent three determinations made at 24-hour intervals for 7 days in two separate experiments.

days caused no further increase in cell number or changes in cyclic AMP levels.

The lack of fluctuation in cyclic AMP levels of L5178Y lymphoblasts in response to alterations in serum concentration may be due to several reasons. These lymphoblasts are malignant cells and therefore have growth characteristics that are different from normal ones. Some factors that may influence cyclic AMP levels are reduced cell-to-cell contact in suspension culture, depletion of one or more nutrients in these rapidly growing cells which may circumvent achievement of saturation density by a physiological mechanism, depletion of one or more serum growth factors, and a defect in the cyclic AMP system. Since Seifert and Paul (8) have shown that 3T3 cells undergo an increase in cyclic AMP levels regardless of whether they are sparse or dense cultures, cell-to-cell contact may not be the most critical

component in regulating cyclic AMP levels. Depletion of a nutrient or serum factor in the lymphoblasts is possible but unlikely, because these cells grew to the same saturation density in either 1%, 5%, or 10% serum. In this context, it is of interest to note that Paul et al. (17) demonstrated that serum growth factor II, which is essential for the growth of 3T3 cells, is not required by transformed cells. Furthermore, Seifert and Paul (8) suggested that when this growth factor II is depleted, 3T3 cells become quiescent and cyclic AMP levels increase. Such a relationship may exist between a serum growth factor, cell density, and cyclic AMP levels in lymphoblasts. In view of the growing accumulation of data relating cell growth and cyclic AMP (1-13), a possible explanation for our results is that the cyclic AMP system is aberrant in these neoplastic cells.

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