Effects of Derivatives of Cyclic 3',5'-Adenosine Monophosphate on the Growth, Morphology, and Gene Expression of Hepatoma Cells in Culture

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SUMMARY

The growth rate of Reuber H35 hepatoma cells in monolayer culture has been shown to be inhibited by N6,O2'-dibutyryl cyclic 3',5'-adenosine monophosphate (DBcAMP). The effect is concentration dependent and readily reversible. Other adenine nucleotides are inactive at concentrations at which DBcAMP is active or cause release of cells into the medium and subsequent cell death at high concentrations. DBcAMP also exhibits similar toxicity at high concentrations, but only DBcAMP and two 8-thio derivatives of cyclic 3',5'-adenosine monophosphate appreciably reduce the growth of attached cells at any concentration. Cells grown in the presence of DBcAMP are larger and also possess characteristic long, narrow processes. HTC hepatoma cells do not exhibit any of these specific responses to DBcAMP, but they do show a nonspecific toxic response with high concentrations of DBcAMP and adenine nucleotides. Among the derivatives of cyclic 3',5'-adenosine monophosphate tested, only those that are effective inducers of tyrosine transaminase are capable of inhibiting the growth of attached H35 cells. The rate of protein synthesis was not inhibited by growth of H35 cells in the presence of DBcAMP and the content of protein was greater in the treated cells. In contrast, DNA synthesis was markedly inhibited shortly after the addition of DBcAMP. It is concluded that DBcAMP and certain derivatives of cyclic 3',5'-adenosine monophosphate are capable of specifically and reversibly inhibiting the growth of H35 hepatoma cells in culture.

INTRODUCTION

The growth and morphology of a number of cultured cell lines have been reported to be influenced by DBcAMP. Exposure of various lines of transformed fibroblasts to DBcAMP has been found to reduce the growth rate and to cause the cells to become more elongated. Similar cellular elongation was found with cultured Chinese hamster ovary cells and human glioblastoma cells after treatment with DBcAMP. In addition, DBcAMP has been reported to induce axon formation in mouse neuroblastoma cells.

Sheppard has also reported that DBcAMP treatment of transformed 3T3 cells leads to a noticeable reduction in agglutinability by wheat germ agglutinin. A similar effect on the restoration of contact-inhibited growth and cell agglutinability was observed in Chinese hamster ovary cells.

Prostaglandins, which are known to stimulate adenylate cyclase activity in vitro in several systems, have also been shown to affect the cellular morphology and agglutinability of Chinese hamster ovary cells and to decrease the growth rate of transformed fibroblasts.

Taken together, these findings suggest strongly that cAMP may play a role in the regulation of the growth and morphological characteristics of certain cells. This paper deals with the results of a study on the effects of DBcAMP on rat hepatoma cell cultures. In the hepatoma cell line Reuber H35, DBcAMP regulates the synthesis of specific enzymes, as is the case in normal liver. In contrast, DBcAMP has been shown to be inactive in influencing enzyme synthesis in the so-called HTC hepatoma cell line. For this reason, the effects of DBcAMP on the growth of Reuber H35 and HTC cells were compared. In the case of H35 cells, effects of DBcAMP on morphology and agglutinability were also examined. A variety of natural and synthetic analogs of 3',5'-cAMP and adenosine nucleotides and prostaglandin E1 have also been examined for effects on these cells. A specific reduction of the growth rate is obtained by exposing H35 cells to DBcAMP and other cAMP analogs which are also effective enzyme inducers. Although the reduction in growth rate did not detectably alter the overall rate of protein synthesis, DNA synthesis was strongly inhibited soon after exposure to DBcAMP.

MATERIALS AND METHODS

The hepatoma cell lines Reuber H35 and HTC were grown as monolayer cultures at 37°C in plastic Falcon flasks with a surface area of 25 sq cm. The standard medium consisted of...
Eagle's basal medium enriched 4-fold with vitamins and amino acids and buffered with Na-tris(hydroxymethyl)methylglycine at pH 7.4. Fetal calf serum and calf serum were both added to a final concentration of 5% unless otherwise indicated. Routine testing for Mycoplasma has been negative.

The number of cells was determined with the aid of a hemocytometer after harvesting with EDTA (1 mM).

The total amount of cell protein was determined after the cells were washed twice with 0.9% NaCl solution, by the method of Lowry et al. (11). The rate of protein synthesis was measured by the incorporation of leucine-3H into hot trichloroacetic acid-precipitable material with the use of filter paper discs according to the method of Mans and Novelli (13). DNA content was measured by the method of Burton (3), with 2-deoxyadenosine as a standard. DNA synthesis was monitored by the incorporation of thymidine-3H into cold trichloroacetic acid-precipitable material as above.

For studies of agglutinability the quantitative agglutination assay of Pollack and Burger (19) was used. Cells were harvested by exposure to EDTA; and the resulting suspensions were centrifuged, washed, and resuspended in 0.9% NaCl solution to a concentration of 10^6 cells/ml. Various concentrations of wheat germ agglutinin were added, and the mixture was allowed to stand at room temperature for 10 min. The concentration indicated is that which produced agglutination of 50% of the cells as viewed microscopically in randomly selected fields. Studies on the induction of tyrosine transaminase were carried out as previously described (1).

All tissue culture components were purchased from Grand Island Biological Co., Grand Island, N. Y., except calf serum, which was obtained from Biogen Co., Denver, Colorado, and fetal calf serum, which was from Colorado Serum Co., Denver, Colorado. DBcAMP (97% purity) was from Boehringer, Mannheim, Germany, 3',5'-cAMP 2',3'-cAMP, and 5'-AMP were from Sigma Chemical Co., St. Louis, Missouri. 8-Thio-cAMP and 8 methylthio-cAMP were a generous gift from Dr. R. K. Robbins (ICN Nucleic Acid Research Institute, Irvine, California). Prostaglandin E, was kindly supplied by Upjohn Co., Kalamazoo, Michigan. Leucine-3H was obtained from New England Nuclear, Boston, Massachusetts, and thymidine-3H was from ICN.

RESULTS

Effect of DBcAMP on the Growth of H35 Cells. H35 Cells cultured under the conditions described in "Materials and Methods" exhibit a logarithmic increase in cell number up to a density of approximately 8 x 10^4/flask. After this point the cells begin to slough off the surface so that a steady state is reached. The doubling time of the cells under these growth conditions is about 40 hr.

The addition of DBcAMP to growing cells results in a marked inhibition of the growth rate, generally 50 to 70% (Table 1). Although no attempt was made to determine the minimum effective concentration of DBcAMP, inhibition is observable at 0.05 mM. The optimal concentration for inhibition of growth is about 0.5 mM; higher concentrations produce nonspecific toxicity as discussed below. The inhibitory effects of 0.5 mM DBcAMP on cell growth at various times after subculture are illustrated in Chart 1. Although inhibition does occur upon addition of DBcAMP at various times after subculture, the inhibitory effect is reduced at higher cell densities. Preliminary results have shown that this is due to the fact that fewer cells are in the DBcAMP-sensitive part of the cell cycle, i.e., the S phase (R. Van Wijk and W. D. Wicks, in preparation).

The phenomenon of contact inhibition does not occur in H35 cells grown normally; after extensive growth on the surface, cells began to pile up and slough off. In the presence of DBcAMP this piling up was not noticeably inhibited.

Chart 2 shows the results of an experiment in which DBcAMP was removed at various times after addition, indicating that the inhibitory effect of DBcAMP on the rate of growth can be readily reversed. A series of experiments was carried out to determine

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### Table 1

Effect of various compounds on the growth rate of H35 cells

<table>
<thead>
<tr>
<th>Compound added</th>
<th>Concentration (mM)</th>
<th>Cells/flask at 6 days [(x 10^4) at the following initial cell densities]</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>4 x 10^4  2.5 x 10^4  1.8 x 10^4  1.5 x 10^4</td>
</tr>
<tr>
<td>DBcAMP</td>
<td>0.05 mM</td>
<td>50  32  14  11</td>
</tr>
<tr>
<td></td>
<td>0.10 mM</td>
<td>43  26  12  11</td>
</tr>
<tr>
<td></td>
<td>0.50 mM</td>
<td>30  12  6  4</td>
</tr>
<tr>
<td></td>
<td>1.00 mM</td>
<td>26b  11</td>
</tr>
<tr>
<td></td>
<td>2.00 mM</td>
<td>25b  11</td>
</tr>
<tr>
<td>3',5'-cAMP</td>
<td>0.5 mM</td>
<td>48  30</td>
</tr>
<tr>
<td></td>
<td>1.0 mM</td>
<td>48b  30</td>
</tr>
<tr>
<td>2',3'-cAMP</td>
<td>0.5 mM</td>
<td>48  30</td>
</tr>
<tr>
<td></td>
<td>1.0 mM</td>
<td>48b  30</td>
</tr>
<tr>
<td>5'-AMP</td>
<td>0.5 mM</td>
<td>51  29</td>
</tr>
<tr>
<td></td>
<td>1.0 mM</td>
<td>48b  29</td>
</tr>
<tr>
<td></td>
<td>2.0 mM</td>
<td>46b  25</td>
</tr>
<tr>
<td>8-Methylthio-</td>
<td>0.5 mM</td>
<td>3</td>
</tr>
<tr>
<td>3',5'-cAMP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-Thio-3',5'-</td>
<td>0.5 mM</td>
<td>9</td>
</tr>
<tr>
<td>cAMP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium butyr-</td>
<td>1.0 mM</td>
<td>11</td>
</tr>
<tr>
<td>Prostaglandin E,</td>
<td>100 µg/ml</td>
<td>11</td>
</tr>
<tr>
<td>Prostaglandin E,</td>
<td>0.5 mM</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>+ DBcAMP</td>
<td>4</td>
</tr>
<tr>
<td>Sodium butyr-</td>
<td>1.0 mM</td>
<td>4</td>
</tr>
<tr>
<td>Prostaglandin E,</td>
<td>0.5 mM</td>
<td>4</td>
</tr>
</tbody>
</table>

* Data in this column were obtained after growth in a medium containing 10% calf serum and 5% fetal calf serum.

* Significant degrees of cell sloughing occurred under these conditions. Counts were corrected on the assumption that the released cells were able to divide at the rate of attached cells.
3',5'-cAMP Analogs and Growth of Hepatoma Cells

whether the effect of DBcAMP was solely an effect on the rate of growth or rather the result of other phenomena such as reduced attachment of cells to the surface and consequent release into the medium, cell death, or some bias in the procedure used for counting.

The number of cells that were released into the medium during a period of 48 hr, i.e., between 2 subsequent changes of medium, was found to be less than 1% of the number of attached cells. This figure was not altered by growth in the presence of 0.5 mM DBcAMP. The number of dead cells as determined by vital staining in situ with the aid of trypan blue during either normal growth or in the presence of 0.5 mM DBcAMP was also found not to exceed 1%. Finally, cell counts were performed in situ by following the growth of designated colonies of cells. The doubling times obtained by this procedure were similar to those obtained by the procedure described in “Materials and Methods.” From these studies it is clear that the effects of DBcAMP are due solely to a lengthening of the doubling time of the cells that remain attached to the surface.

Effects of Other Compounds on the Growth of H35 Cells. The effect of a number of other compounds on the rate of growth were examined (Table 1). Several of these were completely inactive at the concentrations listed: 1 mM sodium butyrate, 0.5 mM 2',3'-cAMP, 0.5 mM 3',5'-cAMP, and 0.5 mM 5'-AMP. At higher concentrations the nucleotides (including DBcAMP) appeared to be toxic since the cells sloughed off the surface and a high percentage of them (~80%) were killed as determined by vital staining.

By assuming that the cells which were released into the medium would have divided at the normal rate if they had remained attached, we could calculate the effective inhibition of growth from counts of attached and sloughed cells at various intervals (Table 1). If the calf serum concentration was raised to 10%, the toxic sloughing response could be eliminated and, except for a slight effect with 5'-AMP, only DBcAMP among the compounds tested was capable of inhibiting the rate of cell growth under these conditions (Table 1).

The effects of 2 new analogs, 8-methylthio-3',5'-cAMP and 8-thio-3',5'-cAMP were qualitatively similar to that of DBcAMP, although there was considerable difference in the degree of growth inhibition. Prostaglandin E1 did not exhibit any effect on the growth of H35 cells, either in the absence of DBcAMP or in its presence (Table 1).

Effects of DBcAMP and Other Compounds on Enzyme Induction in H35 Cells. As has been previously reported, DBcAMP stimulates the synthesis of tyrosine transaminase in H35 cells (1). We have tested a variety of other compounds to see whether there is any correlation between their effectiveness as enzyme inducers and inhibitors of growth.

3',5'-cAMP is only a very weak inducer of the transaminase and 2',3'-cAMP actually produces a slight decline in enzyme activity (Table 2). In contrast, the two 8-thio analogs of 3',5'-cAMP were effective inducers of tyrosine transaminase; the 8-methylthio derivative was the most potent. Prostaglandin E1 led to a decrease in transaminase activity but did not appreciably inhibit the response to DBcAMP. Finally, sodium butyrate did not induce the transaminase. Similar results have also been obtained with phosphoenolpyruvate carboxykinase (W. D. Wicks, unpublished observations). These results suggest that there is a rather strong correlation between the effects of 3',5'-cAMP derivatives on specific enzyme synthesis and inhibition of growth.

Effects on Morphology of H35 Cells. H35 cells exhibit different morphological characteristics during growth in the presence of various inhibitors (Fig. 1). These differences first appear during the early logarithmic phase of growth and persist up to a density of approximately 35 X 10^6 cells/flask. During this period untreated H35 cells have few if any small, narrow processes (Fig. 1A).

In the presence of DBcAMP the cell bodies became enlarged and the long, narrow processes become quite abundant (Fig.
Effects of DBcAMP and other compounds on induction of tyrosine transaminase in H35 cells

Table 2

<table>
<thead>
<tr>
<th>Addition</th>
<th>Final concentration</th>
<th>Tyrosine transaminase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>3',5'-cAMP</td>
<td>0.5 mM</td>
<td>109</td>
</tr>
<tr>
<td>2',3'-cAMP</td>
<td>0.5 mM</td>
<td>84</td>
</tr>
<tr>
<td>8-Thio-3',5'-cAMP</td>
<td>0.5 mM</td>
<td>253</td>
</tr>
<tr>
<td>DBcAMP</td>
<td>0.5 mM</td>
<td>290</td>
</tr>
<tr>
<td>8-Methylthio-3',5'-cAMP</td>
<td>0.5 mM</td>
<td>305</td>
</tr>
<tr>
<td>Prostaglandin E₁</td>
<td>20 µg/ml</td>
<td>68</td>
</tr>
<tr>
<td>DBcAMP + prostaglandin E₁</td>
<td>0.5 mM + 20 µg/ml</td>
<td>261</td>
</tr>
<tr>
<td>Sodium butyrate</td>
<td>0.5 mM</td>
<td>55</td>
</tr>
</tbody>
</table>

Addition of DBcAMP to previously untreated cells leads to these changes in cellular appearance after approximately 1 generation time (~40 hr). By the same token the cells lose most of their processes and become correspondingly smaller within 40 hr after removal of DBcAMP.

The effect of DBcAMP on cell size was even more pronounced when the more active inhibitor of growth 8-methyl-thio-3',5'-cAMP was used (Fig. 1D). The less inhibitory analog 8-thio-3',5'-cAMP exhibited a proportionally smaller effect on cell size (Fig. 1B).

After the normal and inhibited cells approach densities of more than 35 × 10⁶ cells/flask, the morphological differences become less pronounced. Ultimately, all the cells become polygonal and piling up of the cells is observed after prolonged incubation even in the presence of the inhibitor.

Lack of Effect of DBcAMP on HTC Cells. Addition of DBcAMP at 0.5 mM had no detectable effect on the growth and morphology of HTC cells. No growth inhibition was observed in this cell line with other nucleotides such as 3',5'-cAMP, 2',3'-cAMP and 5'-AMP although these compounds, including DBcAMP, were toxic at concentrations of 1 to 2 mM, i.e., caused a reduced attachment of cells to the surface resulting in a high percentage of dead cells.

Prostaglandin E₁ also did not have any effect on the growth characteristics of HTC cells.

In contrast to HTC cells, MH₁ C₁ hepatoma cells do exhibit inhibition of growth rate after exposure to DBcAMP (R. Van Wijk and W. D. Wicks, unpublished observations).

Effects of DBcAMP on Cell Agglutinability. Wheat germ agglutinin was used to probe for possible changes that might have taken place in the plasma membranes as a result of growth in the presence of DBcAMP (8, 22). Untreated H35 cells were found to be only weakly agglutinable; 350 µg of wheat germ agglutinin per ml were required to agglutinate 50% of the cells. Cells grown in the presence of 0.5 mM DBcAMP showed no significant difference in agglutinability by wheat germ agglutinin.

Effect of Growth Inhibitors on the Content and Synthesis of Protein and DNA in H35 Cells. H35 cells grown in the presence of DBcAMP reproducibly contained a larger amount of protein per cell (Table 3). By using the 8-thio analogs, we observed that the amount of protein per cell was correspondingly higher as the degree of growth inhibition increased. As would have been predicted from the greater protein content, the overall rate of protein synthesis was not inhibited by growth in the presence of DBcAMP (Table 3). From these data the total amount of leucine-3H incorporated into protein per cell during one cell cycle can be calculated to be approximately 0.22 cpm, whereas the figure for the DBcAMP-treated cells is about 0.29 cpm, somewhat higher because of the longer duration of the cell cycle (Table 3). This difference is in good agreement with the difference in total protein content per cell. Although the content of protein was greater in DBcAMP-treated cells, there was no detectable...
Table 3

| Cells were grown for 6 days after initial inoculation with 6 x 10^5 cells/flask, and DBcAMP (0.5 mM) was first added 24 hr after subculturing. The doubling time of the cells was determined by constructing a growth curve. After 6 days, 6 flasks of each group were used for determination of the initial rate of leucine-3H incorporation into protein. For these measurements the medium was changed, and 5 ml of growth medium containing 0.9 mM leucine-1H, with or without DBcAMP, were added along with 25 μCi of leucine-1H (36,000 μCi/μmole). The rate of incorporation of leucine-3H into protein was linear for at least 90 min. The same flasks were used for determination of the amount of protein per cell.

<table>
<thead>
<tr>
<th>No. of cells/flask</th>
<th>Doubling time of cells (hr)</th>
<th>Protein (mg/10^7 cells)</th>
<th>DNA (mg/10^7 cells)</th>
<th>Leucine-3H incorporated in 30 min (cpm/cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>70 x 10^5</td>
<td>40</td>
<td>3.0</td>
<td>0.14</td>
</tr>
<tr>
<td>DBcAMP</td>
<td>40 x 10^5</td>
<td>56</td>
<td>4.1</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Chart 3. Effects of DBcAMP on thymidine-3H incorporation in H35 hepatoma cells. DBcAMP (0.5 mM) was added 12 hr prior to the addition of thymidine-3H (1 μCi/flask; 18,000 μCi/μmole). The cells were used 6 days after subculture. Incorporation of thymidine-3H was monitored at the indicated time intervals by the procedure described in “Materials and Methods.” Each point is the average of 2 separate flasks. •, untreated cells; ○, DBcAMP-treated cells.

The difference in the DNA content per cell (Table 3). In contrast, cells exposed to DBcAMP for only 12 hr exhibit a large decrease in the rate of DNA synthesis, as measured by thymidine-3H incorporation (Chart 3).

DISCUSSION

These results have demonstrated that the growth rate of the cultured hepatoma cell line Reuber H35, which is parenchymal in nature, is specifically and reversibly inhibited by DBcAMP. This effect on the increase in cell number was shown to result from an effect on the growth of cells which remain attached to the surface and not to any lethal effects of the cyclic nucleotide. Higher concentrations of DBcAMP were found to be somewhat toxic, as is also the case with other adenine nucleotides, i.e., 3',5'-cAMP, 2',3'-cAMP, and 5'-AMP. Toxicity with these compounds has also been described by Ryan and Heidrick (21) and by Sheppard (22).

This nonspecific toxic effect can be eliminated by growing the cells in a medium containing 10% calf serum instead of the usual 5%. 5'-AMP did exert a weak inhibitory effect at high concentrations, but DBcAMP produced essentially the same inhibition of growth as before. Since 5'-AMP at 2 mM was much less inhibitory than DBcAMP at 0.5 mM, conversion of the cyclic nucleotide to 5'-AMP could not contribute significantly to the observed effect.

In addition, only DBcAMP among these compounds is capable of inducing enzyme synthesis in H35 cells (1). The dose-response curve for inhibition of growth by DBcAMP is remarkably similar to that for enzyme induction (1).

DBcAMP was found to retard only the rate of growth and did not lead to the formation of a clearly contact-inhibited state. In addition, DBcAMP had no effect on cellular agglutinability. However, the agglutinability of untreated H35 cells is very weak when compared to other tumor cell lines (8, 22).

Effects qualitatively similar to those of DBcAMP were obtained with two 8-substituted cAMP derivatives, i.e., 8-methylthio-3',5'-cAMP and 8-thio-3',5'-cAMP. These derivatives have been shown to mimic the effects of 3',5'-cAMP by stimulating the phosphorylation of histone by bovine brain protein kinase (15) and rat liver protein kinase (2). Furthermore, these compounds are stimulators of glycogenolysis in rat liver slices (2). Both analogs are inducers of tyrosine transaminase and the degree of effect correlates roughly with their effect on growth.

This correlation between the effects of the various 3',5'-cAMP analogs on growth and enzyme induction support the view that the inhibition of growth is a highly specific effect related to 3',5'-cAMP and not to some artifact. In addition, the correlation suggests an underlying common denominator for the effects of DBcAMP on these 2 processes.

Measurements of the effect of these growth inhibitors on protein synthesis have shown that there is no decrease in overall rate of this process and as a result, the treated cells contain more protein per cell. This might be the explanation for the enlarged appearance of cells grown in the presence of DBcAMP. The differences in morphology produced by DBcAMP occurred throughout the early logarithmic phase of growth and are not dependent on the cell density, as indicated.
by the observation that DBcAMP causes a similar enlargement in previously untreated cells after only 1 generation time.

The cell density clearly influences cellular morphology at densities above 35 X 10^6 cells/flask even in the presence of DBcAMP. The finding that the action of the inhibitor decreases at high cell densities was also observed with the effects on growth (Chart 1). At present there is no satisfactory explanation for this phenomenon.

In contrast to protein synthesis, incorporation of thymidine-3H into DNA is markedly inhibited during growth in the presence of DBcAMP. A more extensive study on the effect of DBcAMP on the length of the cell cycle has revealed a prolongation of the S phase which is similar to the prolongation of the total cell cycle (i.e., generation time). This effect appears to be due to a nearly immediate partial inhibition of DNA synthesis upon addition of 0.5 mM DBcAMP (R. Van Wijk and W. D. Wicks, manuscript in preparation). Masui and Garren (14) have reported very recently that adrenal tumor cells grown in culture also show a rapid inhibition of thymidine-3H incorporation upon addition of DBcAMP.

DBcAMP did not have any effect on the growth of HTC cells. These results were not unexpected, however, since DBcAMP does not induce tyrosine transaminase in these cells (6). The reason for the nonresponsiveness of HTC cells is not known, but it could be due to a lack of 3',5'-cAMP-dependent protein kinase or some other component(s) involved in the response to elevated levels of intracellular 3',5'-cAMP.

Preliminary experiments have shown that the growth of another hepatoma cell line (MH1C1) is also inhibited specifically by DBcAMP.

Prostaglandin E_1 had no effect on the growth of either H35 cells or HTC cells. Makman (12) observed a stimulation of adenylyl cyclase activity in HTC cells after addition of prostaglandin E_1 to stationary-phase cultures. The lack of effect of DBcAMP on the growth of these cells may therefore be due to a defect in the mechanism by which intracellular 3',5'-cAMP acts on growth, as suggested above. The lack of effect of prostaglandin E_1 on H35 cells is possibly the result of a defect in the adenylyl cyclase of these cells. Adenylyl cyclase activity in H35 cells has been found not to respond to other hormones such as glucagon and isoproterenol in vitro (W. D. Wicks, unpublished observations), and these hormones do not lead to phosphoenolpyruvate carboxykinase induction in H35 cells (1). On the other hand, the fact that Exton et al. (5) have reported that prostaglandin E_1 has no effect on the level of 3',5'-cAMP in perfused rat liver suggests that this compound may not have any effect on 3',5'-cAMP-regulated phenomena in normal liver and as a consequence might not be expected to inhibit the growth of hepatoma cells.

In summary, these results show that the rate of growth of the malignant liver cell line Reuber H35 can be retarded in a specific manner by analogs of 3',5'-cAMP which mimic the action of the cyclic nucleotide in several systems. They further suggest that 3',5'-cAMP may play a role in the regulation of DNA replication. Further studies, including elucidation of the factor(s) controlling the responsiveness of various hepatoma cell lines to 3',5'-cAMP analogs will be required to determine whether alterations in 3',5'-cAMP metabolism play an important role in the process of hepatic oncogenesis.

ACKNOWLEDGMENTS

The H35 cells (H-4-II-E-c-3) and HTC cells were the generous gifts of Dr. V. R. Potter, and Dr. S. Levisohn and Dr. E. B. Thompson, respectively. We would like to thank Dr. J. R. Sheppard for assistance in performing the agglutination studies. The excellent technical assistance of Miss Janet B. McKibbin is gratefully acknowledged.

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