Evidence for cAMP-independent Inhibition of S-phase DNA Synthesis by Prostaglandins*

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Two prostaglandins, prostaglandin E\(_1\) (PGE\(_1\)) and prostaglandin B\(_1\) (PGB\(_1\)), block S-phase DNA synthesis in synchronous cultured baby hamster kidney (BHK) cells. The prostaglandin inhibition of DNA synthesis does not appear to require elevated levels of cAMP. In BHK-21 cells that have been "desensitized" to prostaglandin stimulation of adenylate cyclase and, therefore, have control levels of cAMP, PGE\(_1\) retains its inhibitory effect on the incorporation of tritiated thymidine into DNA. When BHK cells are exposed to PGB\(_1\) (a prostaglandin that does not elicit a cAMP response), DNA synthesis is also blocked. In nonsynchronous cells exposed for 1 h to PGE\(_1\) and then incubated for 1 h with PGE\(_1\) removed, a rebound of DNA synthesis occurs, therefore providing evidence that a transient rise of cAMP in itself is not capable of causing a cascade of reactions that block the synthesis of DNA. In addition, the concentration of PGE required for inhibition of DNA synthesis is significantly less than that required for cAMP generation. Addition of 1 \times 10^{-7} M PGE to BHK cells can be shown to significantly inhibit DNA synthesis within 30 min, with half-maximal inhibition seen at 3 \times 10^{-7} M PGE. Cyclic AMP levels for controls were 4.9 \pm 0.2 and 4.6 \pm 0.1 for 1 \times 10^{-7} M PGE.

These findings suggest that the prostaglandins can act independently of cAMP at physiological concentrations; and, therefore, it is possible that prostaglandins have a physiological role in the control of cell growth during S-phase.

Prostaglandins have previously been shown to cause morphologic changes and retard cell growth in a variety of cells (1-3). One recent publication has demonstrated that analogues of prostaglandins act as inhibitors of tumor growth and suggests using prostaglandins as cancer chemotherapeutic agents (4). In addition, when indomethacin, a potent inhibitor of prostaglandin synthesis, is used to lower synthesis of prostaglandins in tissue culture, there is a stimulation of the proliferation of both HeLa cells (5) and cultures of normal bone marrow colony forming units (6). These findings and others have suggested that prostaglandins have a physiological and, perhaps, a pathological role in the control of cell growth.

Prostaglandins are ubiquitous in nature and several are known to stimulate adenylate cyclase activity, thereby raising intracellular adenosine 3'-5'-cyclic monophosphate levels. It has been suggested that the growth retardation caused by prostaglandins is mediated by an increase in cAMP levels (7-9). The retardation of cell growth by prostaglandins' stimulation of cAMP is a logical suggestion since others have demonstrated that cAMP inhibits cell growth by inhibiting mitosis (10). There is, however, no direct evidence specifically identifying cAMP as the effector of prostaglandin inhibition of growth. In addition, there is little direct evidence indicating which portion of the cell cycle is blocked by prostaglandins.

This study demonstrates that two prostaglandins, one that raises intracellular cAMP and one that does not, both inhibit S-phase DNA synthesis in synchronized cultured cells. Furthermore, we have demonstrated that an increase in cAMP is not necessary for the prostaglandin-induced inhibition of DNA synthesis. These observations add to the existing evidence that prostaglandins per se might play a physiological role in the regulation of the cell cycle.

EXPERIMENTAL PROCEDURES

Reagents—\([^{3}H]\text{-methyl}\text{dThd}\) (20 Ci/mmol) and CAMP radioimmunoassay kits were obtained from New England Nuclear, prostaglandins E\(_1\), F\(_{\alpha}\), and B\(_1\) were obtained from Sigma; dm\(_1\) PGA, and dm PGE, were gifts from Dr. John Pike, The Upjohn Co., MIX was purchased from Aldrich; DDA was purchased from P-L Biochemicals; and MEM with Earle's salts was purchased from the University of California, San Francisco Cell Culture Facility. The media were supplemented with a 1% minimal essential medium vitamin solution, 1% minimal essential medium amino acid solution, 1% Fungizone, 0.5% neomycin solution, penicillin (10,000 units/ml) and 10% tryptose phosphate broth. Hy-Clone FCS (lot 100227 or 100298) was added to the culture media at a final concentration of 0.1, 5, or 10% (v/v).

Cell Culture—BHK-21 cells (a nonmalignant fibroblast cell line) were obtained from the Cell Culture Facility of the University of California, San Francisco. Thyminide incorporation experiments were carried out in 35-mm Petri dishes (Linhbo, Hamden, CT). The cells used for cell cycle experiments were synchronized by serum depletion (11). Briefly, the cells were placed in medium containing only 0.1% fetal calf serum for a period of 36-48 h. Media containing 5% serum were added at the zero time point, after which the cells progressed to S-phase at approximately 14 h and peaking from 16-20 h.

1 The abbreviations used are: dm, dimethyl; PG, prostaglandin; BHK, baby hamster kidney; MEM, minimum essential medium; DDA, 2,5-dideoxyadenosine; FCS, fetal calf serum; MIX, 3-isobutyl-1-methylxanthine.

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Prostaglandin, Indomethacin, DDA, and Control Treatments—Immediately before the experiment, prostataglandins were solubilized in a 24% ethanol stock solution which was diluted with sterile phosphate-buffered saline to achieve a working concentration of 2 mM. 100-μl aliquots of this working solution were added to 1.5 ml of the culture media in each 35-mm Petri dish to yield a final prostaglandin concentration of 0.1 mM. Experiments requiring lower concentrations of prostaglandins were performed by making serial dilutions of the 2 mM working solution. The DDA stock solution made was 3.95 mM in phosphate-buffered saline. On the day of the experiment, the stock solution was diluted to 0.06 mM working solution. The indomethacin concentration was 4 mM in 2% dimethyl sulfoxide in MEM. The final concentration was 3.1 μM with 0.002% dimethyl sulfoxide.

The control vehicle consisted of the highest concentration of ethanol used in the PG treatment, diluted with sterile phosphate-buffered saline. The final in Petri concentration of ethanol never exceeded 0.1%. Aliquots of the vehicle were the same as those used for the PG treatments above.

Measurement of DNA Synthesis—At the specified time points, cells were incubated with [3H-methyl]thymidine (2 μCi/ml of phosphate-buffered saline) for 15 min at 37 °C. Immediately after incubation, the labeled solution was removed and 1.5 ml of ice-cold trichloroacetic acid (5%) were added to each Petri dish. After 30 min, the first trichloroacetic acid was removed and saved for subsequent cAMP assay. The Petri dishes were washed twice more with 1.5 ml of cold 5% trichloroacetic acid and then three more times with 1.5 ml of ice-cold 95% ethanol. The cells were then air dried and solubilized with 2 mM EDTA 1.5 ml of ice-cold 95% ethanol. The cells were then air dried and solubilized with 2 mM EDTA 1.5 ml of ice-cold 95% ethanol. The cells were then air dried and solubilized with 2 mM EDTA 1.5 ml of ice-cold 95% ethanol. The cells were then air dried and solubilized with 2 mM EDTA 1.5 ml of ice-cold 95% ethanol. The cells were then air dried and solubilized with 2 mM EDTA. The labeled solution was removed and 1.5 ml of ice-cold 95% ethanol. The cells were then air dried and solubilized with 2 mM EDTA.

Measurement of cAMP Content—cAMP levels were measured according to the modified method of Rapoport (14) on the same cells of a 30-min exposure to various concentrations of PGE1 on synchronized BHK cells within minutes after addition, shows 22-23%.

Measurement of CAMP Content—cAMP levels were measured according to the modified method of Rapoport (14) on the same cells in which DNA synthesis was determined. Briefly, the tritiated thymidine solution was rapidly aspirated and 1.5 ml of ice-cold 5% trichloroacetic acid 20 ml NaHCO3, 400 μl of this solution was analyzed for tritiated thymidine incorporation in mini vials using 5 ml of Ultrafluor (National Diagnostics, Sommerville, NJ) in a Beckman LS-330 Counter. Counting efficiency of the tritium label in samples was 22–23%.

Protein Determination—Samples were assayed for protein using the method of Lowry et al. (12) as modified by Brown et al. (13).

Table I

| Time after addition (min) | cAMP (pmol/mg protein) |
|--------------------------|------------------------|
| 15                       | Control: 7.9 ± 1.2     |
|                          | PGE: 33.0 ± 0.5        |
| 30                       | Control: 8.7 ± 1.7     |
|                          | PGE: 35.9 ± 2.6        |
| 60                       | Control: 5.2 ± 0.5     |
|                          | PGE: 26.2 ± 1.0        |
| 120                      | Control: 3.4 ± 0.1     |
|                          | PGE: 16.8 ± 1.0        |
| 240                      | Control: 4.0 ± 0.5     |
|                          | PGE: 8.8 ± 0.8         |

*Table II*

Effect of PGE1 concentration on cAMP levels in BHK-21 cells

BHK-21 cells were synchronized by serum depletion for 48 h. The experiment was initiated (t = 0) by adding 5% FCS-MEM. Cells were exposed to PGE1 at 5 h and cyclic AMP levels were measured 30 min later. All results are reported ± S.E. n = 3.

| [PGE] mM | cAMP (pmol/mg protein) |
|----------|------------------------|
| Control  | 7.3 ± 1.1               |
| 0.0001   | 6.33 ± 0.17             |
| 0.001    | 10.13 ± 1.09            |
| 0.01     | 17.51 ± 3.4*            |
| 0.05     | 21.2 ± 2.3*             |
| 0.1      | 44.4 ± 1.7*             |

*p < 0.01.

Fig. 1. BHK-21 cells were synchronized by serum depletion as described in the text. PGE1 (100 μM) was added 2 h prior to study. Duplicate measurement of [3H-methyl]thymidine incorporation into DNA is shown by a solid line for control and a dashed line for PGE1, and cAMP levels in parentheses next to each point in pmol/mg of protein.

of 0.1 mM in order to assure significant elevations of cAMP concentrations in the BHK cells.

Effect of Acute Exposure to PGE1, on DNA Synthesis during the Cell Cycle—We first studied the short term effect (2 h) of PGE1, on DNA synthesis in synchronous cells as illustrated in Fig. 1. In untreated control cells, the serum-depleted synchronous cells were in S-phase DNA synthesis by 14 h after 5% FCS-MEM refeeding. Maximal tritiated thymidine incorporation into DNA was observed in samples at 16 h. Starting at 12 h after refeeding, PGE or vehicle was added sequentially to the synchronized cells to allow a 2-h exposure. At every time point, the 2-exposure to PGE1 effectively blocks S-phase DNA synthesis. The cAMP response is shown inside parentheses by each time point in Fig. 1. At each time point, the PGE1-treated cells showed a marked increase in intracellular cAMP concentration.

We also studied the effect of physiological concentrations of PGE1, which did not increase cAMP during the cell cycle at 16 h. As seen in Table III, both 1 and 5 μM PGE1, inhibit S-phase DNA synthesis. On the other hand, another fatty acid, octanooate, has no effect on S-phase DNA synthesis.

Effect of Chronic Exposure to PGE1, on DNA Synthesis during the Cell Cycle—Since the PGE1 concentration and cAMP levels remain constant at each time point, we determined the effect of chronic exposure to PGE1 on DNA synthesis in cells synchronized in the presence of PGE1. As seen in Table IV, the PGE1 concentration and cAMP levels were similar throughout the experiment.
time course in the previous experiment dictated that cAMP levels would be elevated in the PGE-treated cells, we next examined PGE-treated cells with normal cAMP levels. Within the past few years, several laboratories have demonstrated that when cultured cells are exposed chronically to a variety of hormones or to prostaglandins, the adenylate cyclase response becomes desensitized or "refractory" (15-19). This phenomenon also occurs in BHK cells; within 4-5 h after exposure to PGE, cAMP values then begin to return to control levels, as seen in Table I. We therefore studied [3H]thymidine incorporation during S-phase DNA synthesis in cells chronically treated with PGE. The cells were synchronized by serum depletion for 48 h. The trial was initiated by adding 5% FCS-MEM; 5 h later, 0.1 mM PGE or vehicle was added. As seen in Table IV, at all time points, cAMP levels in cells exposed chronically to PGE are not significantly higher than the nonexposed controls. Nonetheless, prostaglandin E blocks [3H]thymidine incorporation into DNA during S-phase (i.e. from 13-18 h). This finding would suggest that the continued presence of cAMP per se is not necessary for the growth inhibition seen in cells exposed to PGE. Effect of Short Term Rise of cAMP on DNA Synthesis—The possibility that the initial transient rise in cAMP caused by PGE might trigger a chain of events that causes inhibition of DNA synthesis was evaluated in this experiment using nonsynchronous cells. The cells were grown in 35-mm Petri dishes. At the zero time point, the cells were treated either with vehicle or PGE, for 1 or 2 h following which [3H]thymidine incorporation was measured in control and PGE-treated cells. After exposure for 1 h to prostaglandin, the PGE was then removed from one triplicate set of cells.

**Table III**

*Effect of acute exposure of physiological concentrations of PGE on S-phase DNA synthesis*

Synchronous BHK cells were studied 16 h after addition of serum. The cells were exposed to treatment for 2 h before measuring thymidine incorporation. All values are reported as ± S.E.

| Treated thymidine incorporation into DNA | cpm/µg |
|-----------------------------------------|--------|
| Control                                 | 272 ± 10 |
| 1 µM PGE                                | 218 ± 4  |
| 5 µM PGEI                               | 200 ± 3  |
| 5 µM octanoyl                          | 273 ± 13 |

*NS, not significant.

**Table IV**

*Effect of chronic exposure of PGE on DNA synthesis during the cell cycle*

Cells were synchronized by serum depletion for 48 h. The experiment was initiated by adding 5% FCS-MEM. Prostaglandin or control vehicle was added at 5 h after initiation of the experiment. All values are reported as ± S.E. n = 3.

| Time | [3H]Thd incorporation | Cyclic AMP |
|------|-----------------------|------------|
|      | cpm/µg protein | pmol/mg |
| 5    | Control | 88 ± 3 | 4.8 ± 0.2 |
| 13   | Control | 145 ± 20 | 5.6 ± 0.8 |
| 14   | Control | 43 ± 13 | 6.3 ± 0.8 |
| 15   | Control | 173 ± 13 | 3.9 ± 0.2 |
| 15   | Control | 36 ± 3 | 4.2 ± 0.4 |
| 15   | Control | 157 ± 22 | 3.9 ± 0.3 |
| 15   | Control | 32 ± 5 | 4.4 ± 0.4 |
| 16   | Control | 183 ± 34 | 4.0 ± 2 |
| 17   | Control | 37 ± 16 | 4.2 ± 0.3 |
| 18   | Control | 185 ± 18 | 3.8 ± 0.2 |
| 18   | Control | 27 ± 4 | 2.9 ± 0.3 |
| 18   | Control | 259 ± 6 | 5.9 ± 0.7 |
| 18   | Control | 18 ± 1 | 5.1 ± 0.1 |

**Table V**

*Thymidine incorporation into DNA in nonsynchronous cells*

Nonsynchronous cells were exposed to 0.1 mM PGE for 1 or 2 h before measuring DNA synthesis. In one triplicate set, the PGE was removed at 1 h and [3H-methyl]thymidine was measured at the 2-h time point. Cells were in logarithmic growth. n = 6 for 1 h; n = 3 for 2 h.

| Time | [3H]Thd incorporation |
|------|-----------------------|
| h    | cpm/µg |
| 1    | Control | 294 ± 15 |
| PGEI | 243 ± 15³ |
| 2    | Control | 361 ± 21 |
| PGEI | 233 ± 13³ |
| PGEI (R) | 554 ± 16³ |

³p < 0.05.  
³p < 0.001.

**Table VI**

*Effect of PGB concentration on cAMP levels in BHK-21 cells*

BHK-21 cells were synchronized by serum depletion for 48 h. The experiment was initiated at t = 0 by addition of 5% FCS-MEM. Cells were exposed to PGE, at 5 h and cyclic AMP was measured 30 min later. All results are reported ± S.E. n = 3.

**Table VII**

*Effect of PGB concentration on DNA synthesis in BHK-21 cells*

BHK-21 cells were synchronized by serum depletion for 48 h. The experiment was initiated at t = 0 by addition of 5% FCS-MEM. Cells were exposed to PGE, at 5 h and cyclic AMP was measured 30 min later. All results are reported ± S.E. n = 3.

**Fig. 2.** BHK-21 cells were synchronized by serum depletion as described in the text. PGB (100 µM) was added 5 h after serum refeeding. [3H-methyl]Thymidine incorporation into DNA is shown by a solid line for controls and a dashed line for PGB-treated cells.
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nomoglobin suggests that the initial transient rise in cAMP cannot in itself inhibit DNA synthesis and that the continued presence of PGE, is required. In experiments not shown, we removed and replaced the control media and did not see significant increases in DNA synthesis when compared to static control; therefore, the rebound phenomena must be due to the removal of PGE,.

Effect of PGB on DNA Synthesis—A third approach to demonstrate that the effect of prostaglandins on DNA synthesis need not be mediated through cAMP utilizes prostaglandin B 1, a closely related prostaglandin which causes no increase in intracellular cyclic AMP levels at the concentrations studied (20). As shown in Table VII, even at a 0.1 mM concentration of PGB, cAMP levels do not rise above control levels. In this experiment, even though cAMP concentrations are not increased in the prostaglandin-treated cells, PGB still blocks DNA synthesis to the same or greater extent than PGE,,. As seen in Fig. 2, PGB inhibits [3H]thymidine incorporation into DNA during the S-phase of the cell cycle although cyclic AMP levels in the duplicate PGB-treated cells were never significantly higher than controls. This experiment further demonstrates that prostaglandins per se are capable of inhibiting cell growth without increasing cAMP levels.

Effect of Various Prostaglandins on DNA Synthesis—Since both PGE, and PGB, are effective blockers of S-phase DNA synthesis, there was the possibility of a nonspecific PG effect on DNA synthesis. We therefore tested the effect of 2 other prostaglandins. As seen in Table VII, dm PGA, and PGB, both significantly inhibited thymidine incorporation into DNA. On the other hand, PGB, had no significant effect on DNA synthesis. These results would suggest that the inhibitory prostaglandins are acting specifically and not through a general toxic effect.

Effect of Short Term Exposure to Various Concentrations of PGE, on DNA Synthesis and cAMP Levels in the Presence of MIX—As another approach to dissociating the effect of prostaglandins and of cyclic AMP on DNA synthesis, we exposed nonsynchronous cells for a 30-min period to vehicle

### TABLE VII

Effect of various prostaglandins on DNA synthesis

Nonsynchronous BHK cells (n = 3) were seeded 1 day prior to the initiation of the experiment by addition of 5% FCS-MEM with either vehicle or 50 µM prostaglandin. Thymidine incorporation was determined 1 h later. All values are given as mean ± S.E. n = 3.

| Prostaglandin | [3H]Thymidine incorporation into DNA (cpm/µg protein/15 min) |
|---------------|----------------------------------------------------------|
| Control       | 442 ± 13                                                |
| dm PGA,       | 54 ± 4                                                  |
| PGE,          | 243 ± 8                                                 |
| PGB,          | 411 ± 12                                                |

*p < 0.001.

### TABLE VIII

Effect of short term PGE, exposure on thymidine incorporation and cAMP levels in nonsynchronous cells

Nonsynchronous cells in logarithmic growth were seeded 1 day prior to the experiment. The experiment was initiated by the addition of 5% FCS-MEM with either vehicle or PGE,. Thymidine incorporation and cAMP levels were measured 30 min later. All values are given as mean ± S.E. n = 3.

| Treatment             | [3H]Thymidine | cAMP (pm/µg protein) |
|-----------------------|---------------|---------------------|
|Control + MIX         | 840 ± 24      | 5.9 ± 0.3           |
|0.0001 mM PGE, + MIX  | 451 ± 10      | 5.8 ± 0.3           |
|0.001 mM PGE, + MIX   | 359 ± 10      | 10.9 ± 2.0          |
|0.01 mM PGE, + MIX    | 358 ± 10      | 19.6 ± 2.3          |

*All values that are significant are designated as p < 0.001.

or prostaglandin E, plus MIX. The presence of MIX, a known inhibitor of phosphodiesterase, in the media allows an accumulation of intracellular cAMP levels in both PGE, treated and control cells. As seen in Table VIII, we measured both [3H-methyl]thymidine incorporation into DNA and cAMP levels in cells exposed to various concentrations of prostaglandins. Over the range of concentrations examined here, we see a significant rise in cAMP levels only at 10 μM of PGE,. The major point of this experiment is that there is a marked inhibition of DNA synthesis within 30 min after exposure at all concentrations of PGE,. When MIX was added to the BHK cells, the cAMP levels increased about 2-fold (from 3.2 ± 0.3 to 5.9 ± 0.3). Even in the presence of MIX, the inhibition of DNA synthesis is not proportional to cAMP levels nor did it require an increase in cAMP levels.

Effect of Short Term Exposure to Various Concentrations of PGE, on DNA Synthesis and Its Relationship to cAMP Concentration—In a separate set of experiments, we found that the inhibition of DNA synthesis is concentration-dependent over the range from 10^{-5} to 10^{-6} M. Maximal inhibition of DNA synthesis was observed at 1 × 10^{-5} M PGE, at which concentration there was no increase in cAMP levels. As seen in Fig. 3, after only a 30-min exposure, the half-maximal effect of PGE, on DNA synthesis was observed at 3 × 10^{-5} M PGE,.

Effect of PGE, on DNA Synthesis in the Presence of Adenylate Cyclase Inhibitor—As a further method of separating the effect of PGE, and cAMP, we preincubated the cultures of nonsynchronous BHK cells with an adenylate cyclase blocker, DDA (21). We then exposed the cells to 50 µM PGE, for 45 min and measured tritiated thymidine incorporation for the last 15 min. As shown in Table IX, at both 10 and 20 µM DDA, PGE, could still cause a significant decrease in thymidine incorporation into DNA. This would again sug-

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**TABLE IX**

Effect of dideoxyadenosine on DNA synthesis

BHK cells were seeded the day prior to the experiment on the day of the experiment the cells were preincubated in DDA or vehicle for 5 min before addition of PGE,

| Treatment | [3H]Thymidine incorporation into DNA (cpm/µg protein/15 min) |
|-----------|----------------------------------------------------------|
| Control   | 545 ± 13                                                |
| PGE,      | 415 ± 14<sup>*</sup>                                     |
| DDA       | 536 ± 16                                                |
| PGB, + PGE, | 417 ± 11<sup>*</sup>                                    |

<sup>*</sup>p < 0.001 when compared to controls or to cells treated with DDA alone n = 3.
gest a CAMP-independent inhibition of DNA synthesis.

Effect of Indomethacin Inhibition of Prostaglandin Synthesis—Since the work of others (5, 6) has suggested that blocking prostaglandin synthesis with indomethacin might increase DNA synthesis, we added 3 μM indomethacin to the BHK cells for either 2 or 24 h and then measured DNA synthesis for 15 min. As seen in Table X, retarding prostaglandin synthesis with a cyclo-oxygenase inhibitor significantly increased DNA synthesis at both time points. This final experiment gives additional proof to support the suggestion that prostaglandins can play a physiological role in cell growth.

**DISCUSSION**

Many investigators have shown that prostaglandins inhibit cell growth (1-4, 22-24). PGE will inhibit cell growth in L-929 cells (1), aortic smooth muscle cells (23), bone marrow cells (6), Lewis lung carcinoma (4), and B16 melanoma cells (4). Several of the authors have suggested the use of prostaglandins in the treatment of cancer. None of the studies, however, has reported the specific cell cycle location of growth inhibition by prostaglandins. Moreover, the majority of these studies have logically suggested that the prostaglandin-induced growth inhibition is mediated through CAMP since many of the prostaglandins studied have the ability to stimulate adenosylate cyclase activity, thereby causing a rise in intracellular CAMP levels. However, none of the investigations has correlated DNA synthesis with CAMP levels in control and prostaglandin-treated cells.

The possibility that cyclic nucleotides can regulate DNA synthesis and growth is still a highly controversial subject (1, 7-10, 23-29). Some of the studies have investigated the effect of analogues of CAMP added directly to cultured cells in order to define the effect of CAMP on the cell cycle. One study demonstrated that dibutyryl CAMP had no effect on DNA synthesis but instead inhibited mitosis (10). Several studies have suggested that CAMP plays a regulatory role in the cell cycle with G-2 being the most likely site of action (26-29). Therefore, in these studies, CAMP inhibits growth by delaying entry into mitosis, having no direct effect on S-phase DNA synthesis. Moreover, in cells synchronized by serum depletion, the arrest of cell growth is not correlated with an increase in intracellular CAMP (28). It should also be noted that direct addition of cyclic AMP analogues to cultured cells can produce chronic and unphysiological levels of the nucleotide, making it essential that the results obtained be interpreted cautiously (30-33).

In the present study PGE₁, dm PGA₁, and PGB₁ were shown to inhibit DNA synthesis in BHK-21 cells. It is unlikely that this inhibition of growth by the prostaglandins is mediated by cyclic AMP for several reasons. First, in desensitized cells chronically exposed to PGE₁, the prostaglandin had an inhibitory effect on DNA synthesis, even though cyclic AMP levels in the cells are equivalent to or even below the untreated control levels. Second, PGB₁, which has no effect on intracellular CAMP levels, caused the same inhibition of growth as PGE₁. Third, the direct action of prostaglandin was further examined in cells given an acute exposure to PGE for 1 h followed by removal of PGE; the transient rise in CAMP in itself did not result in the inhibition of DNA synthesis seen in PGE-treated cells since removal of PGE allows DNA synthesis to increase above control values. Fourth, addition of an adenylyl cyclase inhibitor did not stop the action of PGE on DNA synthesis. Finally, DNA synthesis was significantly increased in the presence of 3 μM indomethacin. All five major points support our conclusions that the prostaglandins can influence DNA synthesis independent of CAMP.

In this paper, we have studied the effect of PGE on cyclic AMP concentration and DNA synthesis over a wide range of concentrations. Since the initial 1971 observations of Johnson and Pastan (1) used 100 μM PGE₁, we followed their example. Additionally, we selected 100 μM concentrations for our longer term experiments since we are concerned with the well known degradation of PGE in tissue culture. The effect of the prostaglandins is not due to general toxicity of prostaglandin since:

1. the effects are reversible and
2. other prostaglandins do not affect DNA synthesis. Four of the experiments have examined the effect of physiological levels of PGE on DNA synthesis. First, in Table III we see that 1 μM PGE₁ can inhibit S-phase DNA synthesis in synchronized cells while 5 μM octanolate has no effect. In Table VII, we observed that, even in the presence of MIX, physiological levels of PGE inhibited DNA synthesis without elevating CAMP levels. Third, in Fig. 3, we see that inhibition of DNA synthesis is concentration-dependent on PGE and not CAMP. All these studies demonstrate that physiological levels of PGE inhibit DNA synthesis. Finally, we have demonstrated in Table X that 3 μM indomethacin (a blocker of prostaglandin synthesis) significantly increases DNA synthesis over a 24-h period. These data imply that endogenous increases in CAMP levels do not inhibit DNA synthesis when prostaglandins are not present. In addition, the low concentrations of prostaglandins can cause a dramatic decrease in thymidine incorporation without causing any change in intracellular CAMP levels. These observations suggest that the prostaglandins per se affect DNA synthesis and this effect is independent of the prostaglandin-induced increase of CAMP. A second conclusion of this study is that the prostaglandins block cell proliferation by inhibiting S-phase DNA synthesis. By contrast, studies by others have provided evidence that cyclic AMP blocks cell proliferation by inhibiting S-phase DNA synthesis. By contrast, studies by others have provided evidence that cyclic AMP blocks cell proliferation by inhibiting S-phase DNA synthesis.

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**Table X**

*Effect of indomethacin on DNA synthesis*

| Time (h) | Additive | DNA incorporation (cpm/μg protein/15 min) |
|---------|----------|----------------------------------------|
| 2       | Control  | 353 ± 18                               |
| 2       | 3 μM indomethacin | 422 ± 15*                             |
| 24      | Control  | 552 ± 8                                |
| 24      | 3 μM indomethacin | 680 ± 10*                            |

* *p < 0.02.*
* *p < 0.001.*
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