Effects of Prostaglandin on Substrate Uptake and Cell Division in Human Diploid Fibroblasts

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PG (prostaglandin) E_1 inhibits the uptake of uridine, thymidine, 2-deoxy-D-glucose and L-isoleucine into human diploid WI38 fibroblasts. The inhibition occurs within seconds of the addition of the prostaglandin to the culture. PGE_2 , $PGF_{1\alpha}$ and $PGF_{2\alpha}$ behave similarly. Arachidonic acid and 8,11,14-eicosatrienoic acid also decrease uptake in the presence or absence of indomethacin. Other unsaturated fatty acids such as oleic acid, linoleic acid and linolenic acid are essentially inactive. Ricinoleic acid (the 9-hydroxy-oleic acid), however, inhibits uptake to about the same degree, at concentrations similar to those of the prostaglandins. Results indicate that this rapid blockage by the prostaglandins and certain fatty acids is not cyclic AMP-mediated. For example, although $PGF_{1\alpha}$ and $PGF_{2\alpha}$ are much poorer stimulators of cyclic AMP formation than are PGE_1 and PGE_2 , they are nevertheless effective inhibitors of substrate uptake. Adrenaline, a very effective stimulator of cyclic AMP formation in the cells, is not inhibitory. Also, the addition of 8-methylthioadenosine 3':5'-cyclic monophosphate (methylthio cyclic AMP) to the culture does not cause an immediate blockage of uptake. However, 22 h after addition to the culture, methylthio cyclic AMP decreases the uptake of nucleotides into cultures undergoing active cell division, approximately to values found in quiescent cultures. PGE_1 also has this effect on cells undergoing active growth. This gradual decrease in substrate uptake caused by PGE_1 appears to be a separate event from its initial rapid inhibition of uptake.

Molecular transport across the plasma membrane has been proposed by a number of investigators as one of the metabolic parameters participating in the modulation of cell growth. Experimental data substantiate a relationship between substrate uptake and cell division. For instance, Weber & Rubin (1971) reported a decline in uptake of uridine into 3T3 cells, as cell growth became inhibited at a high density of cells. Cunningham & Pardee (1969) demonstrated rapid increases in uptake of uridine and phosphate with serum stimulation of contact-inhibited 3T3 cells. Costlow & Baserga (1974) showed that cyclo-leucine uptake increased 3 h after quiescent WI38 cells were stimulated to proliferate by the addition of fresh serum.

PG (prostaglandin) E_1 can mimic the effect of serum deprivation on substrate uptake. For example, Kram & Tomkins (1973) demonstrated that after serum stimulation, those cultures of Balb/c 3T3 cells that included PGE_1 exhibited a much lower uptake of leucine, uridine and adenosine than did the cultures to which PGE_1 was not added. PGE_1 is capable of reversibly decreasing cell division and the incorporation of [3H]thymidine into DNA in the WI38 human embryo lung fibroblasts (Kurtz *et al.*, 1974). PGE_1 is a potent stimulator of intracellular cyclic AMP

production in the WI38 cells (Kurtz *et al.*, 1974; Kelly & Butcher, 1974; Makman *et al.*, 1973). Froehlich & Rachmeler (1972) demonstrated that the addition of cyclic AMP or 6-*N*,2'-*O*-dibutyryl cyclic AMP inhibits cell division in human lung fibroblasts. We demonstrated that 8-methylthioadenosine 3':5'-cyclic monophosphate (methylthio cyclic AMP), an analogue of cyclic AMP, has an effect very similar to that of PGE_1 on the WI38 cells in culture, in that it also inhibits cell division and the incorporation of [3H]thymidine into DNA (Polgar *et al.*, 1975).

The role of cyclic AMP in the control of cell division is well established, and has been thoroughly reviewed by Pastan *et al.* (1975). Cyclic AMP has also been implicated as a modulator of substrate uptake by several workers (Roller *et al.*, 1974; Hauschka *et al.*, 1972; Grimes & Schroeder, 1973; DeAsua *et al.*, 1974). Although many of the actions of PGE have been cyclic AMP-linked, this compound can also affect the activity of other enzyme systems located in the plasma membrane (Johnson & Ramwell, 1973).

In the series of experiments discussed here, we demonstrate a rapid inhibition by the prostaglandins of the uptake of uridine, thymidine, L-isoleucine and 2-deoxy-D-glucose into the WI38 cells. We explore the

specificity of this action by the prostaglandins. We also examine the role of cyclic AMP, and conclude that the rapid inhibition of substrate uptake by the prostaglandins is not cyclic AMP-related. We find, however, a second gradual inhibition of uptake by PGE₁, an action that appears to be cyclic AMP-linked.

Materials and Methods

Materials

Prostaglandins E₁, E₂, A₁, F_{1α} and F_{2α} were a gift from Dr. J. Pike of Upjohn Co., Kalamazoo, MI, U.S.A. They were placed in aqueous solution with Na₂CO₃. The radioactive substrates [³H]thymidine (20 Ci/mmol), [5-³H]uridine (29.65 Ci/mmol), L-[4,5-³H]isoleucine (65 Ci/mmol) and 2-deoxy-D-[G-³H]glucose (10 Ci/mmol) were obtained from New England Nuclear Corp., Boston, MA, U.S.A. 8-Methylthioadenosine 3':5'-cyclic monophosphate was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. The fatty acids were obtained from Applied Science Laboratories, State College, PA, U.S.A. The radioimmunoassay for cyclic AMP was obtained from Schwarz/Mann, Orangeburg, NY, U.S.A.

Cell culture

WI38 human diploid fibroblasts were obtained from Dr. L. Hayflick at passage 12, and were handled as described by Kurtz *et al.* (1974), with some modifications. Experiments were conducted only on cultures in passage 18–30. The cells were grown in 80 cm² bottles for maintenance and passage, and in 20 cm² Petri dishes for incubation (Falconware; Fisher Scientific Co., Medford, MA, U.S.A.). The cultures were kept at 37°C in a humidified incubator and gassed with CO₂+air (5:95). The growth medium was composed of basal medium, Eagle's medium (Grand Island Biological Co., Grand Island, NY, U.S.A.) (90%, v/v), foetal calf serum (10%, w/v), streptomycin (50 i.u./ml) and penicillin (50 i.u./ml). The foetal calf serum was virus-screened (Grand Island Biological Co.). The cells were judged to be mycoplasma-free by the method of Schneider *et al.* (1974). The cultures were put into a parasynchronized state by allowing them to become quiescent in the medium containing 10% foetal calf serum. This occurred a few days after contact density (day 10), with one medium change at day 5 after plating at a density of 1 × 10⁴ cells/cm². Quiescent cell cultures were stimulated to divide by replacement of old culture medium with fresh culture medium. Under these conditions, maximum DNA increase, as determined by DNA assay, was obtained at 22 h after stimulation.

Cell-growth activity of the cultures was followed by cell counts and total DNA determination by the diphenylamine method of Burton (1956).

Cell incubation and assay for cyclic AMP content

Cellular cyclic AMP content was determined as described by Kurtz *et al.* (1974). Incubations were initiated by the addition of a tenfold concentration of 'hormone' solution to the cell culture. At the end of the incubation, the medium was removed by aspiration, and the cell cultures were fixed with 1 ml of 70% (w/v) HClO₄, containing a trace of cyclic [³H]AMP. The cell debris was removed from the dish by scraping, and the supernatant containing the cyclic AMP was obtained by centrifugation at 1000g for 30 min. After neutralization with KOH, centrifugation, and chromatography on a Dowex 50 column (6 mm × 35 mm) with water as eluent, the supernatant containing the cyclic AMP fraction was assayed for the cyclic nucleotide content by radioimmunoassay (Steiner *et al.*, 1969).

Substrate uptake

Uptake of substrate was determined by the method of Cunningham & Pardee (1969), with slight modifications. For determinations of L-isoleucine and 2-deoxy-D-glucose uptake, the cultures were washed and then incubated in phosphate-buffered saline (0.1 M-NaCl/0.05 M-NaH₂PO₄). Incubations with [³H]thymidine and [5-³H]uridine were similar, except that the cells were incubated either directly in the culture medium or in phosphate-buffered saline, as indicated. To stop the incubation, the incubation medium was quickly removed by aspiration. The cultures were then washed four times with ice-cold 0.9% NaCl and fixed with 10% (w/v) trichloroacetic acid. The whole fixing procedure was done on ice, and took less than 30 s.

Acid-soluble and -insoluble radioactively labelled fractions were separated by centrifugation. Radioactivity in the acid-insoluble material was determined by either solubilization in 5% trichloroacetic acid at 70°C for 20 min or trapping on a Millipore filter. Radioactivity was determined in Aquasol (New England Nuclear Corp.). A Packard Tri-Carb liquid-scintillation counter was used. The quenching in various fractions showed negligible variation.

Results

PGE₁ inhibits the uptake of uridine (Fig. 1) and thymidine (Fig. 2) into WI38 cells in culture. The influx of uridine and thymidine into the acid-soluble cell fraction is suppressed within 10 s of the addition

Fig. 1. Effect of PGE₁ on uridine uptake in quiescent and growing WI38 cultures

A group of quiescent cultures was stimulated to divide with fresh culture medium. Duplicate plates of quiescent and stimulated cultures were then incubated with 1 μ M-uridine, containing 25 μ Ci of [5-³H]uridine per plate. The abscissa represents time of incubation. All points are mean values of duplicate determinations. Variation between duplicates did not exceed 10%. Q, quiescent cultures; Q + E₁, corresponding cultures treated with PGE₁ simultaneously with uridine incubation; S, quiescent cultures stimulated with fresh culture medium for 22 h; S + E₁, corresponding cultures treated with PGE₁ simultaneously with uridine incubation. (a) and (b) Acid-soluble cell fraction; (c) acid-insoluble cell fraction.

Fig. 2. Effect of PGE₁ on thymidine uptake in quiescent and growing WI38 cultures

Quiescent cultures and quiescent cultures that were stimulated to divide with fresh culture medium were incubated with 1 μ M-thymidine, containing 2.5 μ Ci of [³H]thymidine per plate. The length of time of each incubation is shown on the abscissa. PGE₁ (50 μ M, final concn.) was added to the cultures simultaneously with the start of the incubation. Values indicate the means of duplicate plates. Variation between duplicate values never exceeded 8%. Q, quiescent cultures; Q + E₁, quiescent cultures treated with PGE₁ at start of incubation; S, quiescent cultures stimulated with fresh culture medium for 22 h; S + E₁, 22 h-stimulated cultures treated with PGE₁ at start of incubation with label. (a) and (b) Acid-soluble cell fractions; (c) acid-insoluble cell fractions.

of PGE₁ (Figs. 1*a* and 2*a*). This suppression is then reflected in decreased incorporation of label into the acid-insoluble cell fraction (Figs. 1*c* and 2*c*). Quiescent cultures, stimulated to divide by the addition of fresh culture medium (containing 10% fresh serum), exhibit an increase in uptake of both uridine and thymidine when determined at 22h after serum stimulation. The suppression of uptake by PGE₁ is, however, the same in both the quiescent and stimulated cultures. After the initial inhibition, we found no further decrease for at least 90min after the addition of the prostaglandin. PGE₁ also inhibits the uptake of L-isoleucine and 2-deoxy-D-glucose into the acid-soluble cell fraction, as demonstrated in Figs. 3 and 4. The inhibition is also detectable within seconds after the addition of the prostaglandin.

At 300 μ M, prostaglandins E₂, F_{1 α} and F_{2 α} possess approximately the same inhibitory activity as PGE₁ (Fig. 5). PGA₁, however, is considerably less effective. At lower concentrations, PGE₁ is more effective than prostaglandins E₂, F_{1 α} and F_{2 α} .

A lack of correlation between the rapid effect of the prostaglandins on substrate uptake, and their effect on cyclic AMP concentrations and cell division is demonstrated in Table 1. PGF_{1 α} and PGF_{2 α} inhibit approx. 60% of 1 μ M-thymidine uptake, but have negligible effect on cyclic AMP concentrations or cell division. PGE₁ and PGE₂, on the other hand, inhibit uptake to approximately the same degree as the F-type prostaglandins, but they also increase cyclic AMP concentrations and inhibit cell division. PGA₁ is a poor inhibitor of uptake, but effectively

Fig. 3. Effect of PGE₁ on L-isoleucine uptake in quiescent cultures

Quiescent cultures were incubated with 0.5 μ M-L-isoleucine, containing 2.5 μ Ci of L-[4,5-³H]isoleucine per plate. The incubation was done in phosphate-buffered saline. PGE₁ (50 μ M, final concn.) was added to the cultures simultaneously with the isoleucine. The abscissa represents the time of incubation. The ordinate represents radioactivity in acid-soluble fraction. Mean of duplicate plates was used for each time-point. Variation between duplicate values did not exceed 10%. Q, quiescent cultures; Q + E₁, quiescent cultures treated with PGE₁ at start of incubation.

Fig. 4. Effect of PGE_1 on uptake of 2-deoxy-D-glucose in quiescent and growing cultures

A group of quiescent cultures as stimulated to divide with fresh culture medium. The stimulated and quiescent cultures were incubated with 0.5 mM-2-deoxy-D-glucose, containing 2.5 μ Ci of 2-deoxy-D-[3 H]-glucose. The incubations were done in phosphate-buffered saline. The stimulated cultures were incubated either 10 min or 22 h after stimulation. PGE_1 (50 μ M, final concn.) was added to the cultures either simultaneously with the incubation or with the fresh culture medium and retained for 22 h. The abscissa represents time of incubation. Each point represents a mean of duplicate cultures. Variation between duplicate values did not exceed 10%. Q, quiescent cultures; Q + E₁, corresponding cultures treated with PGE_1 simultaneously with 2-deoxy-D-glucose incubation; S, quiescent cultures stimulated with fresh culture medium for 22 h; S + E₁, corresponding cultures treated with PGE_1 simultaneously with 2-deoxy-D-glucose incubation.

increases cyclic AMP concentrations and inhibits cell division.

The inhibitory effect of PGE_1 is substrate-concentration-dependent (Table 2). The effect of 50 μ M- PGE_1 decreases with increasing uridine concentrations. At 100 μ M-uridine, 50 μ M- PGE_1 inhibits only 13% of uridine uptake.

As Table 3 shows, ricinoleic acid, 8,11,14-eicosatrienoic acid and arachidonic acid also suppress the uptake of uridine at 50 μ M concentrations. Indomethacin, at concentrations known to suppress the conversion of arachidonic acid into PGE_2 in fibrosarcoma cells in culture (Levine, 1972), has no effect on the uptake of uridine. This concentration of indomethacin also does not prevent the inhibitory action of arachidonic acid. Other unsaturated fatty acids (oleic acid, linoleic acid and linolenic acid) are essentially inactive at 50 μ M concentrations.

Fig. 5. Effect of various prostaglandins and adrenaline on uridine uptake

Each plate was incubated with 1 μ M-uridine, which included 2.5 μ Ci of [3 H]uridine. All the prostaglandins and adrenaline were added to the cultures simultaneously with the introduction of the label. The lowest concentrations were 5 μ M. The cultures were incubated for 2 min. Each value is a mean from duplicate cultures. Variation did not exceed 10%. Uptake (% of control value) represents (uptake in given incubation/uptake in control) $\times$ 100.

Unlike the prostaglandins tested, methylthio cyclic AMP does not decrease uptake immediately after addition (Table 4). In fact, no effect on the influx of thymidine or uridine is noted, even at 3 h after the addition. Methylthio cyclic AMP (1 mM) does, however, diminish substrate uptake in cultures when it is retained for 22 h. In cultures stimulated with serum in the presence of methylthio cyclic AMP, the uptake of uridine and thymidine is increased only slightly after 22 h, as compared with cultures stimulated with serum in the absence of methylthio cyclic AMP.

PGE_1 has an effect similar to that of methylthio cyclic AMP on actively dividing cultures; this is illustrated in exponentially growing cells (Fig. 6). PGE_1 causes an immediate decrease in uptake of [3 H]thymidine into acid-soluble cell fraction; then further decreases occur at 12 and 22 h after PGE_1 addition. PGE_1 has a similar effect on quiescent cultures which are stimulated with fresh culture medium.

Table 1. *Effect of prostaglandins on substrate uptake, cyclic AMP concentration and DNA synthesis in WI38 cells in culture*

Cells were plated at a density of 1×10^5 cells/20 cm² dish. The cultures were fed and brought to quiescence as described in the Materials and Methods section. The quiescent cultures were stimulated to divide with fresh culture medium (basal medium, Eagle's medium + 10% foetal calf serum). All culture conditions were produced in duplicate. The final prostaglandin concentration was 50 μ M. To determine the effect on cell division, the prostaglandins were added to the quiescent cultures simultaneously with the fresh medium. DNA content was determined at 48 h after stimulation, and was compared with the DNA content of the quiescent cultures. Substrate uptake was determined with 0.1 μ M-[³H]thymidine, with a 2 min incubation at 24 h after stimulation with serum. The PG was added simultaneously with the [³H]thymidine. Percentage uptake represents total uptake (c.p.m.) in treated cultures divided by total uptake (c.p.m.) in untreated cultures. Cyclic AMP concentrations were determined at 10 min after the addition of the prostaglandin.

Additions	Uptake (%)	Cyclic AMP (stimulated/basal)	DNA (μ g/48h)
Fresh medium	100	1	+6
+PGE ₁	30	37.5	+2.2
+PGF _{1α}	40	1.2	+5.2
+PGA ₁	80	17.0	+0.6
+PGE ₂	40	33.6	+2.8
+PGF _{2α}	39	1.5	+5.4

Table 2. *Effect of PGE₁ on uridine uptake at various uridine concentrations*

Quiescent cultures were treated with 50 μ M-PGE₁ (final concentration). PGE₁ was added to the cultures simultaneously with the given concentration of uridine, containing 2.5 μ Ci of [³H]uridine per plate. The cultures were incubated in phosphate-buffered saline for 2 min. Percentage uptake represents total uptake (c.p.m.) in treated cultures divided by total uptake (c.p.m.) in corresponding untreated cultures. All percentages are means of duplicate cultures. Variation between duplicate samples did not exceed 10%.

Uridine concentration (μ M)	Uptake (%)
100	83
10	60
1	30
0.1	20

Discussion

We have previously demonstrated that PGE₁ inhibits cell division and elevates cyclic AMP concentrations in WI38 cells in culture (Kurtz *et al.*, 1974; Polgar *et al.*, 1974). In the present report, we

Table 3. *Effect of various fatty acids on uridine uptake*
Each plate of quiescent cells was incubated with 1 μ M-uridine (2.5 μ Ci of [³H]uridine) and the given concentration of fatty acid. The indomethacin concentration was 50 μ M. The cultures were incubated for 2 min. Uptake (% of control) represents (uptake in given incubation/uptake in control) $\times$ 100. To ensure inhibition of prostaglandin synthesis, the indomethacin (50 μ M) was added to the cells 3 h before the addition of arachidonic acid.

Fatty acid (50 μ M)	Uptake (% of control)
None	100
Oleic acid	96
Linoleic acid	95
Linolenic acid	94
Arachidonic acid	68
Arachidonic acid + indomethacin (50 μ M)	66
8,11,14-Eicosatrienoic acid	48
Ricinoleic acid	36
PGE ₁	38

demonstrate that the prostaglandins can also affect substrate uptake in the cells. The action of the prostaglandins on substrate uptake appears to be dual: (1) rapid, occurring immediately at the time of addition to the culture; (2) gradual, commencing several hours after the addition and progressing over a span of hours.

The rapid inhibition, which takes place within 10 s of addition, appears not to be cyclic AMP-mediated, since: (1) increasing PGE₁ concentrations beyond 50 μ M, the point of its maximum stimulatory activity on cyclic AMP formation (Kurtz *et al.*, 1974), continues to increase its effect on uptake; (2) 50 μ M-PGF_{2 α} stimulates cyclic AMP formation only minimally in WI38 cells, yet it inhibits uptake to approximately the same degree as prostaglandins of the E type; (3) adrenaline increases cyclic AMP formation (Kelly & Butcher, 1974; Makman *et al.*, 1973), but does not cause rapid suppression of uptake; (4) methylthio cyclic AMP (1 mM) does not cause rapid suppression of uptake.

The results obtained suggest, instead, that the prostaglandins affect substrate transport directly. The very rapid manner of the inhibition suggests action at the cell surface. The action of these compounds on uptake resembles that of various competitive inhibitors of transport (Plagemann & Richey, 1974). For example, as the concentration of uridine is increased, the effect of PGE₁ on uridine uptake decreases; also, the uptake of 1 mM-uridine can be almost entirely abolished with 300 μ M-PGE or -PGF.

Plagemann & Sheppard (1974) have shown, similarly, that PGE₁ inhibits substrate uptake rapidly after addition to Novikoff hepatoma cells. Sheppard & Plagemann (1975) confirmed our observation that

Table 4. Effect of methylthio cyclic AMP on uptake of uridine and thymidine

All the cultures were incubated for 2min in phosphate-buffered saline with 1 μ M-uridine or -thymidine containing 2.5 μ Ci of corresponding labelled nucleotides per plate. The quiescent cultures were treated as indicated: methylthio cyclic AMP-0, methylthio cyclic AMP (1 μ M) was added simultaneously with the label; methylthio cyclic AMP-3, methylthio cyclic AMP was retained in the culture for 3 h before the 2min incubation with thymidine or uridine; methylthio cyclic AMP-22, methylthio cyclic AMP was retained in the culture for 22 h before incubation with the labelled substrate; S, quiescent cultures were stimulated with fresh culture medium for 22h before incubation; S+ methylthio cyclic AMP-0, corresponding cultures to which methylthio cyclic AMP was added simultaneously with the introduction of labelled substrate; S+ methylthio cyclic AMP-22, corresponding cultures where methylthio cyclic AMP was retained in the culture for the length of the stimulation, 22h. All values are means of duplicate determinations. Variation between duplicate values did not exceed 10%.

Additions	³ H]Thymidine		⁵ - ³ H]Uridine
	Acid-soluble fraction	Acid-insoluble fraction	Acid-soluble fraction
None	4708	489	25480
Methylthio cyclic AMP-0	—	—	24984
Methylthio cyclic AMP-3	—	—	24620
Methylthio cyclic AMP-22	3944	324	—
S	21616	6366	64392
S+ methylthio cyclic AMP-0	21850	—	—
S+ methylthio cyclic AMP-22	9124	1404	17964

Fig. 6. Effect of PGE₁ on thymidine uptake in exponentially growing cultures

PGE₁ (50 μ M, final concn.) was added to a group of cultures undergoing active growth at day 5 after plating. Plates of PGE₁-treated and untreated cultures were then incubated with 1 μ M-thymidine, containing 2.5 μ Ci of [³H]thymidine per plate. Incubations were done simultaneously with the addition of PGE₁, and at 12 and 24 h after the addition of PGE₁. The length of time of incubation is indicated on the abscissa. The ordinate represents radioactivity in acid-soluble fractions. All points were done with duplicate cultures and represent mean values. Variation between duplicate values did not exceed 10%. ■, +E₁-0, cultures treated with PGE₁ and incubated with thymidine simultaneously; □, -E₁-0, corresponding cultures not treated with PGE₁; ▲, +E₁-12, cultures treated with PGE₁ for 12h before incubation; △, -E₁-12, corresponding cultures not treated with PGE₁; ●, +E₁-24, cultures treated with PGE₁ for 24h before

this rapid inhibition does not correlate with an increase in cyclic AMP concentration. In their latest experiments, they show that PGE₁ and theophylline affect uptake in this manner in a number of cell lines, including 3T3 and SV40-transformed cells.

The presence of hydroxy groups on fatty acid molecules may affect their activity. This is suggested by results in Table 3 showing ricinoleic acid to be a much more effective inhibitor of uptake than oleic acid. The hydroxy group on C-11 of the prostaglandins also appears important, as illustrated in Fig. 5, which shows that PGE and PGF are much more effective in decreasing uptake than is PGA.

These experiments also stress that care must be taken not to over-interpret labelling experiments as absolute indicators of cell division. For example, in this instance the limiting of entrance of labelled thymidine into the cell, and, therefore, its incorporation into DNA did not reflect the actual cell division that was occurring (Table 1).

The experiment with methylthio cyclic AMP (Table 3) suggests that the gradual inhibition of uptake by PGE₁, which we observed in actively dividing cell cultures and in quiescent cell cultures activated to growth by serum, is a cyclic AMP-linked event. These experiments also indicate that this inhibition may be associated with the decrease in cell division caused by PGE₁. A similar action by PGE₁, that of inhibiting serum-stimulated uptake of uridine in 3T3 cells, was also noted by Kram & Tomkins (1973). This activity by PGE₁ may indeed be a part of a synchronized effect on the metabolic events leading to

incubation; ○, -E₁-24, corresponding cultures not treated with PGE₁.

the initiation or termination of cell division. It is not clear, as yet, what, if any, physiological role exists for the rapid inhibition of substrate uptake by the prostaglandins.

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