# Effects of Gas Tension on Epidermal Keratinocyte DNA Synthesis and Prostaglandin Production*

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The gas phase partial pressure of $O_2$ ($PO_2$) overlying mouse keratinocyte cultures controls the rate of DNA synthesis of these cells by an undefined mechanism. In these studies, both $PO_2$ and $PCO_2$ tensions overlying primary cell cultures were varied within the physiologic range. The prostaglandin (PG) production of cells grown under several varying gas tensions was then determined using radioimmune assay. The cultures were grown under a $PO_2$ of either 7.5% (physiologic for in vivo epidermis) or 21% (atmospheric; culture conditions and wound healing) for 5 days. The $PCO_2$ was either 5 or 10%, 2 $CO_2$ tensions routinely used in tissue culture studies. DNA synthesis was quantitated using [$^3$H]dThd uptake into DNA and autoradiography. The $PGE_2$ and $PGF_{2\alpha}$ syntheses by the cultures over specific time periods were determined. Changing the $PO_2$ from 21 to 7.5% decreased the rate of DNA synthesis, while PG production remained constant. When the $PCO_2$ was varied from 5 to 10%, keratinocyte DNA synthesis remained unchanged but PG production was markedly stimulated. The $PCO_2$ effect on PG production was greatest at the highest oxygen tension. The data indicated that under this set of variables, PG production by keratinocytes is not directly related to the rate of DNA synthesis of the cells. Apparently the amount of oxygen in the gas phase can have a permissive effect on epidermal keratinocyte PG production. *J Invest Dermatol 86:177–180, 1986*

Disorders of cellular proliferation and arachidonic acid metabolism often occur simultaneously in the skin. Arachidonic acid, an essential free fatty acid, is a prescursor for a number of active lipids, the eicosanoids. Most cellular arachidonic acid is bound in membrane phospholipids. The enzyme phospholipase $A_2$ acts to form free arachidonic acid in a calcium-dependent step of eicosanoid metabolism. Free arachidonic acid is then metabolized in skin via the enzyme lipoxygenase to form HETEs, diHETEs, and leukotrienes or by cyclooxygenase to form prostaglandins (PGs). A number of these arachidonic acid metabolites are mediators of cutaneous inflammation [1–3].

In fatty acid-deficient rats, epidermal proliferation is greatly increased while PG synthesis is greatly decreased [4,5]. However, in inflammatory diseases of the skin such as psoriasis, hyperproliferation is present and the epidermis has increased PG synthesis [6]. In a number of studies, drugs which are known inhibitors of phospholipase $A_2$ and of cyclooxygenase inhibited in vitro epidermal keratinocyte DNA synthesis [3,6,7]. Thus the interaction between arachidonic acid metabolism and the rate of epidermal proliferation is still unclear.

To determine whether keratinocyte PG production and entry into DNA synthesis are related, the effect of oxygen tension on these 2 functions was assessed. Physiologic changes in culture oxygen partial pressure ($PO_2$) were used because $PO_2$ controls keratinocyte DNA synthesis without affecting the differentiation of the cultures [8]. Also, since molecular oxygen is a cosubstrate for PG synthesis [2,3,6], shifts in $PO_2$ could directly affect PG synthesis. The results indicated that changes in keratinocyte PG synthesis are not involved in the effect of $PO_2$ on DNA synthesis. However, the percent $PO_2$ could control the magnitude of increases in PG synthesis seen when another stimulus of arachidonic acid metabolism was applied.

## MATERIALS AND METHODS

**Reagents and Animals** Neonatal mice were obtained from the BALB/c colony at the University of Michigan Medical School. Powdered Medium 199 (M-199, growth medium), fetal bovine serum (FBS), penicillin and streptomycin, and Dulbecco's phosphate-buffered saline (PBS) were obtained from Flow Laboratories, Inc. (Rockville, Maryland). Unlabeled antibodies to $PGE_2$ and $PGF_{2\alpha}$ were from Upjohn, Inc. (Kalamazoo, Michigan). Tritiated labeled standards were obtained from New England Nuclear (Boston, Massachusetts). Ficoll 400 and trypsin (2× crystallized) were purchased from Sigma Chemical Co. (St. Louis, Missouri). Corning plastic products were used throughout for cell culture preparation and growth. All other chemicals were reagent grade.

**Primary Neonatal Mouse Epidermal Cultures** Primary neonatal mouse keratinocyte cultures were prepared from BALB/c mice by the method of Marcelo et al [10] using trypsin separation of full-thickness skin. The cell fraction was plated in M-199 with 10% FBS at a density of $2 \times 10^6$ cells per 35-mm Petri dish, and grown at 32°C. All experiments were done with 5-day-old cultures, which are confluent but not fully stratified.

The cells were grown in a Heraeus type B 5060-EK/$O_2$ incubator which maintains the internal $PO_2$ and $PCO_2$ by continuous monitoring. The $PO_2$ and $PCO_2$ of the atmosphere inside the incubator were also verified by routine analysis of the medium for blood gases. Additionally, a polarographic electrode was used

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Abbreviations:
FBS: fetal bovine serum
M-199: Medium 199
PG: prostaglandin
$PO_2$: oxygen partial pressure
MEM: Dulbecco's modified Eagle's medium
RIA: radioimmune assay

to confirm the $PO_2$ of the medium before and after each experiment; the pH of the medium, an indirect indicator of $PCO_2$, was recorded.

When the $PCO_2$ of the gas phase was increased to 10%, an equivalent amount of sodium bicarbonate was added to the medium to maintain the pH at 7.5 which changed the Na ion content of the medium by approximately 10% and resulted in a Na ion concentration similar to that of Dulbecco's modified Eagle's medium (MEM). Without this adjustment, the pH of the medium fell to 7.1. Medium was preincubated and gassed for 24 h prior to the experiment to achieve complete gas equilibrium.

**Radioimmune Assay (RIA)** Cultures were rinsed twice with gas-equilibrated PBS, and 2 ml of equilibrated growth medium were added to each 35-mm dish. After 30, 45, 75, and 120 min or 24 h, the medium was collected and assayed by RIA for $PGE_2$ and $PGF_{2\alpha}$. Our earlier studies using mass spectrophotometry and thin-layer chromatography [3] and high-pressure liquid chromatography [11] showed that $PGE_2$ and $PGF_{2\alpha}$ are the major PGs synthesized by these cells. The medium was spun at 258 *g* for 7 min to remove particulate matter and the samples were frozen at −70°C until assayed. Radioimmune assay was done using antibodies specific for $PGE_2$ [12] and $PGF_{2\alpha}$ [13]; these antibodies showed low cross-reaction with a number of related PGs. RIA has been shown to accurately reflect indomethacin-inhibitable PG synthesis in this culture system [3,6].

**Protein, DNA, and DNA Synthesis Assays** The protein of the cultures was determined by the Lowry protein assay [14]. DNA synthesis was quantitated using autoradiography and the incorporation of [$^3$H]thymidine ([$^3$H]dThd) into DNA as previously described [10]. DNA was assayed by the method of Burton [15]. Data were calculated as counts per minute of $^3$H per microgram of DNA and expressed as percent control. Triplicate or quadruplicate Petri dishes were used per time point and n = 3.

For autoradiography, cells grown on Lux plastic coverslips were labeled for 18 h with 1 μCi/ml of [$^3$H]dThd and processed as previously described [10]. Fifty fields per coverslip were counted. The data were expressed as the mean ± SEM of labeled cells per 100 cells. Slight variations in [$^3$H]dThd transport by the cultures would not affect the results since all labeled nuclei were counted. Other variables such as radioactive background, cell plating density, and exposure time were tightly monitored and were internally corrected using values from control cells grown under nonexperimental conditions.

Possible changes in plating efficiency and cell turnover were assessed by counting the number of cells shed by the control and experimental cultures.

Growth of the keratinocytes in $10^{-7}$M indomethacin for 5–7 days had little or no effect on the rate of DNA synthesis of the cells (data not presented).

**Statistical Analysis** The data were presented as mean ± SEM. The significance was determined using the Student's *t*-test.

## RESULTS

**Effect of $PO_2$ of 7.5 and 21% on Keratinocyte DNA Synthesis and PG Production** Epidermal keratinocyte cultures grown under a $PO_2$ of 7.5% (physiologic) [8] were compared with cultures grown under a $PO_2$ of 21% (atmospheric). The $PCO_2$ was 5% and the medium pH was 7.5. A $PO_2$ of 7.5% was used in these experiments because our earlier studies show that this tension is nontoxic to the cells and is a reasonable approximation of the basal layer oxygen tension in the neonatal mouse [8,16]. The results of these experiments are presented in Table I and Fig 1. The keratinocytes grown under a $PO_2$ of 7.5% showed one-third the rate of DNA synthesis as the control ($PO_2$ = 21%) cultures; both methods for determination of DNA synthesis gave the same results (Table I). Although the rate of DNA synthesis changed, no significant difference in PG production between the control and experimental cultures was observed (Fig 1). Several data bases were used to compare the data: PG/Petri dish, PG/μg protein, and PG/μg DNA. Since all the data gave similar results, only the PG/Petri dish data were present in Fig 1.

**Table I.** Effect of $PO_2$ on Keratinocyte DNA Synthesis

| | 21% $PO_2$ and 5% $PCO_2$ | 7.5% $PO_2$ and 5% $PCO_2$ | % Control |
|---|---|---|---|
| cpm/μg DNA | 1260 ± 287 | 489 ± 134 | 38.8 |
| $^3$H-labeled cell per 100 cells | 10.3 ± 1.3 | 3.7 ± 0.8 | 35.9 |

Cells grown under a $PO_2$ of 21% showed a 3-fold greater rate of DNA synthesis than did those grown under a $PO_2$ of 7.5%. The 21% $PO_2$ value is the control value.

**Effect of 5 and 10% $PCO_2$ on Keratinocyte DNA Synthesis and PG Production** In these experiments, a $PO_2$ of 21% (atmospheric) was used. The gas phase $CO_2$ was either 5 or 10% throughout the experiment and the pH of the medium was 7.5. The results are shown in Table II and Fig 2. As presented in Table II, the rate of DNA synthesis of the cultures was unaffected by the change in $PCO_2$. Growth of the cells under 10% $PCO_2$ did not change the total protein or DNA content of the dishes, nor did it affect the number of cells shed from the culture surface. As presented in Fig 2, changing the $PCO_2$ from 5–10% resulted in a statistically significant 4- to 6-fold increase in $PGE_2$ and $PGF_{2\alpha}$ production by the keratinocytes at all the time points studied.

The $PO_2$ of the gas atmosphere was then adjusted to 7.5% and the cultures were grown under either 5 or 10% $PCO_2$. The data, presented in Fig 3, showed that under the 7.5% oxygen atmosphere the stimulation of PG production by 10% $PCO_2$ was greatly decreased. Under the 7.5% $PO_2$ growth conditions, PG production was increased 1.2- to 2-fold by 10% $PCO_2$, an effect which at a number of time points was not statistically significant. This was in marked contrast to the results from experiments done at a $PO_2$ of 21% where marked changes in PG production were seen. As presented in Table III, growth of the cells under 10% $PCO_2$ did not change the rate of DNA synthesis of the keratinocytes when compared with the 5% $PCO_2$ control cultures.

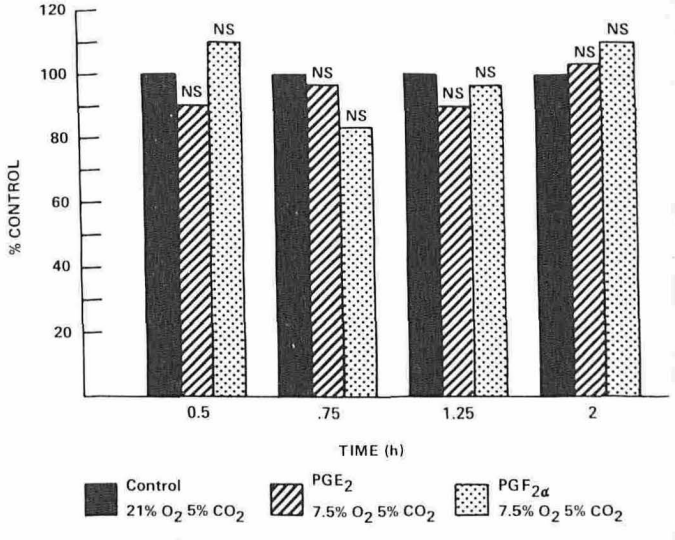


**Figure 1.** Lack of effect of changes in $PO_2$ on total synthesis of $PGE_2$ and $PGF_{2\alpha}$ per Petri dish (5% $PCO_2$). *NS* = not significant. $PGE_2$ and $PGF_{2\alpha}$ production was linear the first 2 h of the assay period. Data are presented as percentage of control.

**Table II.** Effect of $PCO_2$ on Keratinocyte DNA Synthesis ($PO_2$ = 21%)

| | 21% $PO_2$ and 5% $PCO_2$ | 21% $PO_2$ and 10% $PCO_2$ | % Control |
|---|---|---|---|
| cpm/μg DNA | 1080 ± 113 | 1053 ± 118 | 97 |
| $^3$H-labeled cells per 100 cells | 7.3 ± 1.9 | 8.3 ± 1.8 | 117 |

No significant change in DNA synthesis resulted from keratinocyte growth under increased $CO_2$ tension. The 21% $PO_2$ value is the control value.

## DISCUSSION

The inhibition of epidermal keratinocyte DNA synthesis by growth under low $PO_2$ is a physiologic, nontoxic effect [8]. No significant change in cell number, in the amount of total protein, or in total DNA per culture results when the $PO_2$ of the gaseous environment is varied from 5–21%. Neither culture morphology nor the sodium dodecyl sulfate gel electrophoretic pattern of the cellular proteins is altered by cell growth in low $PO_2$.

Our present study showed that keratinocyte PG production was also unchanged when cell DNA synthesis was modified by changing the $PO_2$. However, PG production by these cultures was increased many-fold by adjusting the $PCO_2$, without any concurrent effect on keratinocyte DNA synthesis. The effect of changes in $PCO_2$ on PG production was unexpected, and no reasonable explanation for this phenomenon is presently available. However, the results did suggest that PG production and keratinocyte entry into DNA synthesis were being controlled independently, thereby indicating that a change in PG synthesis was not a mediator of the $PO_2$ proliferative effect.

Although changes in $PO_2$ did not directly affect PG production by the keratinocyte cultures, the gas phase oxygen tension did modify the quantity of PGs produced under the stimulatory effect of increased $PCO_2$. Keratinocytes grown under a $PCO_2$ of 10% produced much more PGs when the $PO_2$ was 21% than when it was 7.5%. The data suggested that this "permissive" effect of 21% $PO_2$ on $CO_2$-induced PG production is independent of a direct $PO_2$ effect on keratinocyte DNA synthesis. It is probable that the increased PG production by the keratinocytes grown under 21% $PO_2$ and 10% $PCO_2$ resulted from oxygen-mediated changes in the function of the cells. Hypothetically, the 21% $PO_2$ effect could result from the increase in oxygen concentration in

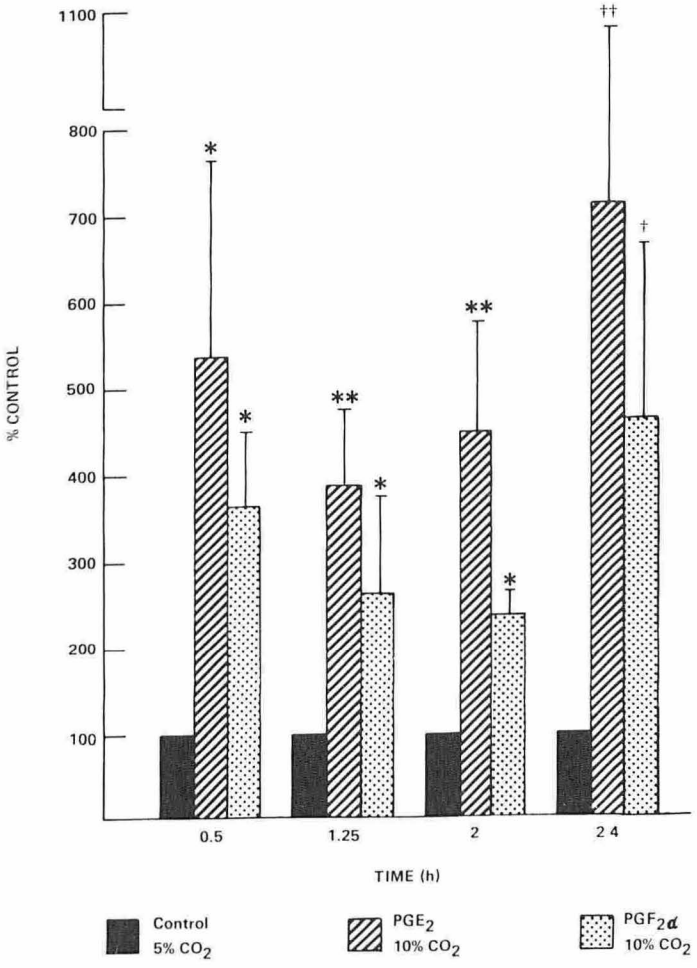


**Figure 2.** Effect of $PCO_2$ on total synthesis of $PGE_2$ and $PGF_{2\alpha}$ per Petri dish (21% $PCO_2$). $\star p \geq 0.025$, $\dagger p \geq 0.01$, $\ddagger p \geq 0.05$, $\star\star p \leq 0.01$. The data were also significant using the PG/μg protein and PG/μg DNA data bases.

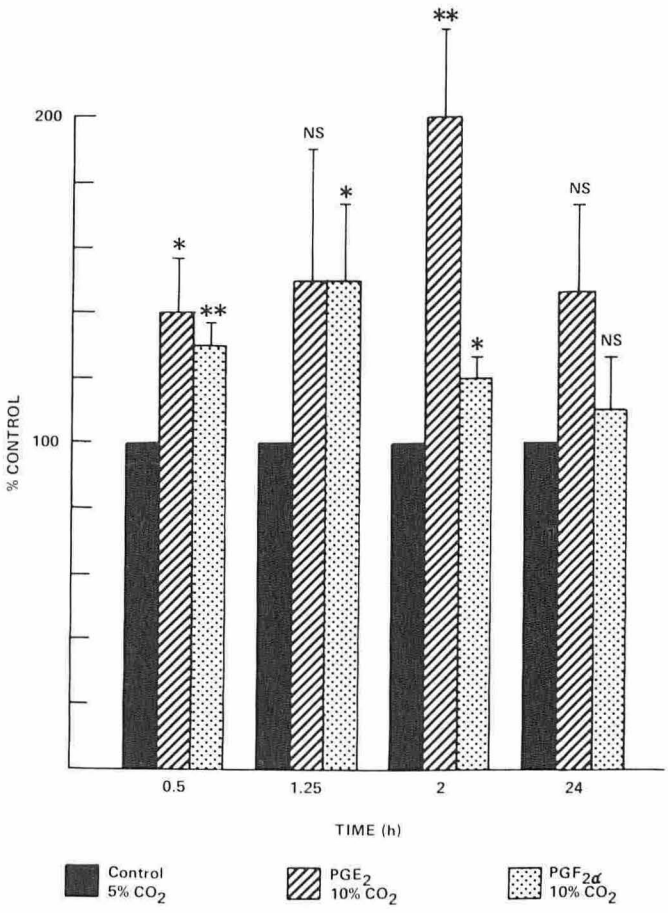


**Figure 3.** Effect of $PCO_2$ on synthesis of $PGE_2$ and $PGF_{2\alpha}$ per Petri dish (7.5% $PO_2$). $\star p \geq 0.025$, $\star\star p \geq 0.01$. *NS* = not significant.

**Table III.** Effect of $PCO_2$ on Keratinocyte DNA Synthesis in Cultures Grown Under a $PO_2$ of 7.5%

| | 7.5% $PO_2$ and 5% $PCO_2$ | 7.5% $PO_2$ and 10% $PCO_2$ | % Control |
|---|---|---|---|
| cpm/μg DNA | 606 ± 66 | 716 + 45 | 118 |
| $^3$H-labeled cells per 100 cells | 2.2 + 0.43 | 1.8 + 0.6 | 82 |

No significant difference in the rate of DNA synthesis was observed. The 5% $PCO_2$ value is the control value.

itself since it is a cosubstrate for cyclooxygenase. Two moles of oxygen are required for the synthesis of each mole of PG; thus oxygen concentration could become a limiting factor when cells maintained under a 7.5% $PO_2$ were stimulated. Presumably, sufficient oxygen was present in the unstimulated state to meet substrate requirements. It is also possible that some change in cellular metabolism associated with the increased DNA synthesis seen at 21% $PO_2$ or with the increased $PO_2$ in itself might result in increased synthesis of cyclooxygenase or phospholipase $A_2$, or in the activation of these enzymes, as has been recently suggested by Jonas and Needleman [17].

Similar events could be occurring in in situ epidermis. The uppermost layer of the epidermis, the stratum corneum, is impermeable to oxygen so that a basal layer $O_2$ of 5–7.5% is maintained [18]. Because atmosphere $PO_2$ is 21%, a breach of the epidermal barrier which would occur in disease states or wound healing [19] could result in an increase in the basal layer $PO_2$. Theoretically, this could alter the rate of epidermal proliferation. Since PGs are involved in the inflammatory changes which occur after wounding [20], any modification of PG production by oxygen-mediated events or an increased proliferative rate could be important to the healing process. Our studies showed that an increase in basal layer $PO_2$ and/or in epidermal proliferation may permit enhanced PG production in response to a stimulus. This same mechanism may play a role in inflammatory processes.

In hyperproliferative inflammatory epidermal diseases such as psoriasis [3,6,20], $PGE_2$ and $PGF_{2\alpha}$ are increased by 40 and 80%, respectively, in psoriatic plaque tissue [6]. Our data suggest that the increased PG production observed in this tissue is not essential to the hyperproliferative state of the psoriatic epidermis. It is possible, however, that hyperproliferation has a permissive effect, so that a normally occurring physiologic stimulus could result in an exaggerated production of PGs. Another possibility is that the incomplete differentiation of, or local increase in microcirculation [21] of the psoriatic epidermis could result in a basal layer increase in oxygen tension. This event could lead to hyperproliferation with subsequent exaggerated PG synthesis in response to physiologic stimuli. Further studies are planned to test this hypothesis and to delineate the role of the lipoxygenase products, the HETEs, diHETEs, and leukotrienes, in epidermal keratinocyte function.

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