Prostaglandin E₁ Effects on Epidermal Cell Growth "in vitro"

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Summary. The effect of prostaglandin E₁ (PGE₁) on growth of mouse epidermis has been studied in vitro. Preliminary experiments showed that PGE₁ 20 μg/ml caused an increase in H3-thymidine labelling index after 1 hr and an increase in thickness and epidermal cell counts after 4 hrs culture. The uptake of H3-thymidine and change in total amount of DNA and protein were then determined using isolated cell cultures. Incubation of cell cultures with PGE₁ 0.1--20 μg/ml for 1 and 4 hrs resulted in increased uptake of H3-thymidine, DNA synthesis and protein synthesis.

Prostaglandin-synthesising activity has been demonstrated in the epidermis of several species including frog [3], rat [8] and man [2,10]. A possible role for prostaglandin in the control of epidermal cell replication has been suggested [4, 5]. We have therefore investigated the effect of prostaglandin E₁ on epidermal cell replication and macromolecular synthesis in vitro using embryonic mouse skin.

Materials and Methods

Animals. Specimens of embryo epidermis was obtained from Swiss-Webster albino mice. The embryos were removed by caesarian section, one or two days before the estimated time of birth. Each culture tube contained epidermis obtained from one embryo. Approximately 300 specimens were grown in vitro for experiments described in this paper.

Cell Culture. The back skin was removed and trypsinized for 20 min to separate the epidermis from the dermis. The epidermis was then incubated again at 37°C with 0.5%/o Trypsin (Burroughs Wellcome Ltd) in phosphate-buffered saline for about 1 hr until the tissue structure disappeared. The separated epidermal cells were
then added to the culture medium. The culture medium used contained 85\% Dulbecco's modified Eagles medium (Burrough's Wellcome Ltd). 15\% Foetal Bovine Serum (Flow Laboratories). Penicillin G 200 units/ml and Streptomycin 200 \mu g/ml. The medium containing the suspended cells was mixed thoroughly to achieve an even distribution of cells. Two millilitres of this suspension were then transferred to culture tubes. That the suspension had been divided evenly between the tubes was suggested by determination of the protein content of the aliquots which varied by less than 10\%. The cultures were then put into a roller apparatus at 16 r.p.m. and incubated at 37\(^\circ\)C.

**Organ Culture.** This method was basically the same as that for cell culture except that, instead of trypsinization, the tissue was cut into pieces approximately 2 \times 2 mm and placed in the culture medium.

**Autoradiography.** A 1 hr pulse of 1 \mu Ci of H\textsuperscript{3}-Thymidine per ml of medium was added to the organ cultures. Thymidine (methyl-H\textsuperscript{3} specific activity: 152 mCi/mmol) was obtained from Radiochemical Centre, Amersham.

After fixation, the tissue was cut into 5 \mu sections and exposed for 2 weeks using Kodak stripping film. For calculation of the mitotic index 500 basal cells were counted from each edge inwards.

**Measurement of Cell Number and Epidermal Thickness in Organ Culture.** After culturing, the explant was stuck dermis-down on a piece of cardboard to keep it straight for fixation and stained with Hematoxylin and Eosin. Thickness measurements were made on a paper copy drawn by projecting the section onto a piece of paper and drawing around the epidermis. The number of epidermal cells per unit of length of full thickness epidermis was counted using a micrometer attachment on a microscope.

**Determination of Amount of DNA in cell culture.** After culturing the medium was spun off and cells washed three times with buffer. Two ml 0.5\% cold perchloric acid were added and left for 4 hrs. DNA was extracted twice with 1 ml N perchloric acid at 70\(^\circ\)C. After each extraction the extract was centrifuged and the supernatant fractions combined. The diphenylamine method was used for DNA estimation \[1\], and the optical density of the perchloric acid extract of DNA incubated for 18 hrs with diphenylamine reagent was read at 600 \mu.

**Uptake of H\textsuperscript{3}-Thymidine.** The uptake of H\textsuperscript{3}-Thymidine was measured by liquid scintillation counting. A 1 hr pulse of 1 \mu Ci H\textsuperscript{3}-Thymidine per ml of medium was added to the cell culture. After culturing DNA was extracted as described above. 0.5 ml of the extract was mixed with 3 ml of Triton-X-100 (Packard Instrument Corporation) to which was added 7 ml of the scintillation mixture (6 g/l PPO and 0.5 g/l Dimethyl POPOP in toluene). The channels-ratio counting method was used; efficiencies of 40\% were obtained using a Packard-Tricarb counter.

**Determination of Protein.** After extraction of DNA the epidermal cells were washed in buffer and taken up in N NaOH overnight. The Lowry method was used \[6\]. The optical density of the resulting blue solution was read at 500 \mu.

**Results**

**The Effect of PGE\textsubscript{1} on Epidermis in Organ Culture**

**Histology.** In preliminary experiments the effect of prostaglandin E\textsubscript{1} on the thickness of epidermis in mouse skin organ culture was studied. Skin slices incubated for 4 hrs with PGE\textsubscript{1} 20 \mu g/ml showed a significant increase of 12\% (P < 0.025) in the thickness of the epidermis compared