Response of Human Primary Foreskin Cells to a Cell-Extract Obtained from Skin[1]

Mammalian tissues contain homeostatic control mechanisms which regulate cell multiplication, and one such mechanism has been shown to function in vivo in skin via a mitotic inhibitory material produced by skin[2]. This material has been partially purified and is thought to be a basic glycoprotein which functions in complex with adrenalin and possibly hydrocortisone, and is tissue specific and species nonspecific[3–5].

Considerable work has been done to demonstrate this inhibitory effect on in vivo epidermal mitoses in the mouse[6,7] and rabbit[8]. With the exception of IVERSEN's[9] organ culture investigation on mitotic activity in human skin, no experiments have examined the effects of skin extracts on human primary skin cells in culture. In this regard, cultures of human primary foreskin cells have been used to investigate the effects of an extract obtained from skin on the growth and division of skin cells. As primary cell cultures often retain the basic characteristics of their tissues of origin[10,11], such a system might be useful in assessment of cell growth influencing factors in extracts of skin.

Procedure. Monolayer cultures of human primary foreskin cells obtained from the Naval Biological Laboratory, Alameda, California were used in all experiments. Cells were cultivated in Eagle's Minimum Essential Medium (with Earle's salts), supplemented with 10% fetal calf serum and antibiotics (penicillin: 50 units/ml; streptomycin: 50 µg/ml). Cultures were maintained at 37 °C and supplied with fresh medium every 3rd day. Stock primary cultures were passaged between the 2nd and 3rd day after reaching confluency into 250 ml Falcon tissue culture flasks at a seeding concentration of $7.5–10.0 \times 10^5$ cells per flask with 25 ml of medium ($3–4 \times 10^4$ cells/ml). For all experiments, cultures were seeded into 30 ml Falcon tissue culture flasks at a concentration of 3×10^5 cells per flask in 8 ml of medium (3.75×10^4 cells/ml). The percentage of cells in mitosis were determined from visual counts of mitotic cells present in 15 randomly selected fields using inverted phase contrast microscopy. Cells were released from the Falcon tissue culture flasks with a 0.03% trypsin (in calcium-magnesium free phosphate buffered saline) solution. Cell numbers and cell volumes were determined with a Coulter Counter (Coulter Electronics, Hialeah, Florida) connected to a multichannel pulse-height analyzer. Trypan blue dye exclusion was used to test viability.

The experimental medium contained skin extract at a concentration of 1.0 mg protein/ml and adrenalin at a concentration of 2.5×10^{-6} mg/ml. Skin extract was prepared from rats in the following way: immediately after sacrifice the hair was plucked, the skin dissected, cut into small strips, frozen with dry ice, and processed through a Universal meat grinder. Cold physiological

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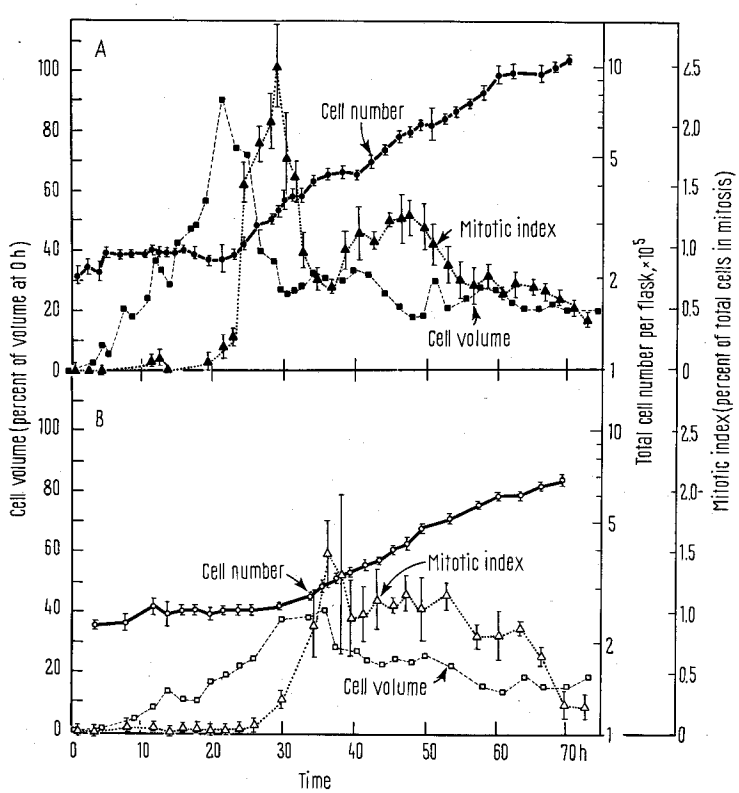

A. Changes in cell volume, mitotic index and cell number for control human foreskin cells. Data points indicate the mean and standard deviation obtained from the measurement of 5 flasks per time interval.

B. Changes in cell volume, mitotic index and cell number of human foreskin cells grown in medium containing skin extract (1.0 mg protein/ml) and adrenalin ($2{\cdot}5 \times 10^{-6}$ mg/ml). Data points represent the mean and standard deviation obtained from the measurement of 5 flasks per time interval.

saline was added to the initial homogenate, which was further processed through a Waring Blender to a fine consistency, placed in a Serval refrigerated centrifuge and spun for 30 min at 13,500 *g* (22,000 *g*), at 4 °C. The surface lipid material and the cellular residue were discarded. The remaining yellowish solution was filtered through a 0.45 micron millipore filter, lyophilized and stored at 0 °C until added to the experimental medium. The protein concentration of the extract was determined from LOWRY [12] protein analysis with human serum albumin (Cutter Laboratory, Berkeley, California) as protein standard. In these extracts the ratio of carbohydrate to protein was approximately 1:50 as determined by an anthrone test with dextrose as standard.

Results and discussion. In Figure A, changes in mitotic index, cell volume and cell number are shown for normal control cultures of human primary foreskin cells. It may be seen that the cell volume reaches a maximum at about 21 h after seeding. Following this cell volume maximum, one sees the initial increase in cell number, which begins to rise about 24 h after seeding. Also, at 24 h the mitotic index begins to rise dramatically, coming to a maximum halfway through the initial increase in cell number, and decreasing rapidly as this increase in cell number levels off to a plateau, having completed the first division cycle. This plateau occurs between 35–40 h after seedings, after which time a second cell division wave commences, although the effect is not as marked. This damping of the response is also shown by the mitotic index and cell volume curves. In all control growth experiments, we have found this highly predictable initial temporal surge of cell division. This 'pseudosynchronous' pattern can probably be attributed to our standard method of serial cultivation.

Cell cultures initiated with experimental medium containing skin extract and adrenalin exhibit a definite increase of 8 h in the lag period preceeding the first wave of cell division. This longer lag period is apparent in all three cell parameters studied as shown in Figure B. In comparison with the control cell growth parameters, the initial step increase in cell number has been eliminated, and the well defined peaks of mitotic index and cell volume have been reduced in amplitude and sharpness. These changes strongly indicate a decrease in the degree of 'pseudosynchrony' as compared to control cultures. Growth parameters of cell cultures initiated with adrenalin alone were not different fron control cultures. Also, cultures treated at various times (32–42 h after seeding) during active cell division with biochemical extracts obtained from leg muscle alone (1.0 mg protein/ml), leg muscle (1.5 mg protein/ml) plus adrenalin, or lung extract (1.5 mg protein/ml) plus adrenalin showed no difference from control cultures. Growth inhibition was seen with cultures treated only with skin extract (1.0 mg protein/ml) and this inhibition was slightly potentiated by the combination of skin extract with adrenalin. This data indicates that while adrenalin will potentiate the effect, the active component is contained in the skin extract.

Our skin extract can be considered patent, as mouse in vivo experiments indicate approximately a 16% depression in ear epidermis mitotic index as compared to control animals after i.p. injections of 1.0 mg protein/g of animal. Also, in preliminary experiments investigating possible cellular modes of action of skin extract, effects on rat liver mitochondrial respiration and oxidative phosphorylation in vitro were examined. No effects of extract were found, suggesting that the effect of mitotic inhibition is not via inhibition of cell respiration or oxidative phosphorylation.

Although our extract has definite effects on the mode of growth of human primary foreskin cells in tissue culture, further work in extract purification and mode of action is needed.

Zusammenfassung. Der Einfluss eines biochemischen, zellfreien Hautextraktes auf menschliche Vorhautzellen wurde in Kultur geprüft. Allgemeine Hemmung von Zellwachstum, Zellvermehrung, Zellvolumen und Mitoserate sprechen für aktiven Bestandteil des Extraktes.

W.A. SCHILLING, G. MAGILEN and J.T. LEITH

Donner Laboratory of Medical Physics, University of California, Berkeley (California 94720, USA), 19 July 1971.

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The Effect of Pretreatment with Rat Placental Homogenate on Subsequent Pregnancy in the Rat

There is increasing interest in the problem of intrauterine fetal malnutrition in human pregnancy as a result of accumulating evidence which suggests that severely affected infants may suffer physical and/or mental retardation in later life [1–6]. Its cause is not known.

The present paper describes an attempt to produce fetal growth retardation in pregnant rats using an immunologic method which involves repeated injection of homologous rat placental homogenate in Freund's adjuvant.

Materials and methods. Three groups of rats were investigated: 1. Rats injected with homologous placenta in Freund's adjuvant before pregnancy. The preparation of homogenate and injection was as described by OKUDA and GROLLMAN [7]. A regime of 8 bi-weekly injections was administered i.p. under ether anaesthesia to a group of 25 rats, after which the animals were mated. Rats weighed from 153 to 196 g at the start of the experiments.

2. Rats injected with homologous placenta in Freund's adjuvant during pregnancy. Twice weekly injections were given i.p. under ether anaesthesia during the 3 weeks of gestation.

3. Control rats. These were normal untreated rats which were not tampered with in any way before or during pregnancy.

Rats were mated, proteinuria determined and placenta and fetal tissues obtained by methods described elsewhere [8].

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