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Effect of Epidermal Chalone on Human Epidermal Mitotic Activity *in vitro*

EXTRACTS of vertebrate epidermis contain an antimitotic chemical messenger, called the epidermal chalone, which seems to be important in the control of epidermal mitosis¹. Although the epidermal chalone is known to be tissue-specific, it is neither species nor class-specific. Chalone extracted from human skin has been found to be active on mouse epidermis², but no experiments have yet tested the effects of skin extracts on human epidermis.

Pieces of human skin, about 10–15 cm long and 1–1.5 cm wide, were obtained during abdominal operations, and immediately immersed in cold sterile saline. The subcutaneous adipose tissue was cut away, and the epidermis with the upper part of the corium was removed from the skin with a Castrovieijo keratome. These slices (0.6 mm thick) were floated on the surface of Hanks balanced salt solution, and a piece of sterile paper to which the skin easily adhered was placed underneath. The paper was then laid on "black paraffin" (50 per cent carbo medicinale and 50 per cent paraffin, melted together in a Petri dish and heat sterilized) and cut into small pieces, about 4 × 4 mm. When floated on Hanks solution once more, the paper sank and the skin floated. The pieces of skin were placed on a stainless steel grid in plastic culture dishes filled with sufficient nutrient medium to cover the grids, but not the skin.

The nutrient medium consisted of Parker's solution 199, human AB serum, and chicken embryo extract (6 : 2 : 2). Neomycin was used as an antibiotic. The dishes were placed in an airtight box which was gassed with a mixture of 5 per cent carbon dioxide and 95 per cent oxygen. The pieces of skin were incubated for 4 days at 37° C; the medium was changed after 2 days. This procedure was found to be necessary, for only after 4 days did the epidermal mitotic rate recover sufficiently for reliable counts to be made. By this time, in almost all pieces of skin, the epidermis had moved round to encapsulate the dermis², while at the edges there was a thick rim of degenerate (parakeratotic) epithelial cells.

The pieces of skin were divided into two groups, A and B, the latter being the control group. Both groups were given 'Colcemid' (0.01 ml. of a 1 per cent solution/2.5 ml. of medium). We carried out some preliminary experiments to determine whether the epidermal chalone could exert any effect without pretreatment with adrenaline. We found no difference between cultures treated with "liver chalone" (discussed later) or with epidermal chalone. This confirmed the observation that epidermal chalone has no specific antimitotic effect when adrenaline is not present¹. One hour after the start of 'Colcemid' treatment, all pieces of skin were therefore given a 5 min wash in a Petri dish containing 0.5 µg of adrenaline/ml., and in experiment 9 hydrocortisone (2.5 µg/ml.) was added

with the adrenaline. Then the pieces of skin were returned to their culture dishes containing the same medium with 'Colcemid'. To group A skins, skin extracts from either mice or pigs were added. In the first three experiments group B skins were given one drop of saline, while in the last six experiments with group B skins extracts of pig liver (prepared exactly like the mouse skin extracts) were added. The epidermal chalone was extracted from pig skin by N. V. Organon (Oss, The Netherlands) and was partly purified after precipitation with 70–80 per cent alcohol. Chalone from mouse skin was prepared in our laboratory and was not purified, but only lyophilized. Each culture was given a dose of chalone (from skin or from liver) corresponding to a protein content of 5 mg/ml. Four hours after the initial addition of 'Colcemid', that is, after 3 h of chalone action, all the pieces of skin were fixed in Bouin's solution for at least 4 h. After embedding in paraffin, sections 5 µ thick were cut. Twenty-four explants were used in each experiment, twelve in group A and twelve in group B. In each group 16 × 40 vision fields were counted. In each experiment the numbers of mitoses have thus been scored among 48,000 cells in the chalone treated groups, and 48,000 cells in the control groups.

Table 1. EFFECT OF CHALONE ON HUMAN SKIN *in vitro*

| Source of chalone | Experiment No. | Mitotic count per 1,000 cells after 1 h 'Colcemid', 5 min adrenaline and then 3 h 'Colcemid' and chalone | | | Percentage depression of mitotic rate |
|-------------------|----------------|--|----------------------|--------------------------|---------------------------------------|
| | | Saline added* | Liver extract added* | Epidermal chalone added* | |
| Pig | 1 | 7.4 ± 0.70 | — | — | 58 } Mean 54 |
| | 2 | 7.8 ± 1.00 | — | 4.5 ± 0.60 | |
| | 3 | 10.1 ± 1.27 | — | 5.1 ± 0.66 | |
| | 4 | — | 8.0 ± 0.77 | 5.7 ± 0.57 | 29 } Mean 40 |
| | 5 | — | 5.4 ± 0.74 | 2.7 ± 0.43 | |
| Mouse | 6 | — | 4.1 ± 0.44 | 2.2 ± 0.31 | 46 } Mean 51 |
| | 7 | — | 7.7 ± 0.85 | 3.5 ± 0.62 | |
| | 8 | — | 6.8 ± 0.78 | 3.4 ± 0.68 | |
| | 9† | — | 5.4 ± 0.59 | 2.6 ± 0.24 | 52 |

* Values are mean mitotic counts from a total of 48,000 cells scored ± standard errors of the mean using the number of explants ($n=12$) for calculation.

† In this experiment hydrocortisone was added together with the 5 min adrenaline wash.

Results are shown in Table 1. Extracts of liver depressed the human epidermal mitotic rate slightly, but not significantly. Compared with the control groups, chalone extracted from pig and mouse skin depressed the human mitotic rate by about 50 per cent. These observations confirm previous observations that watery extracts of epidermal tissue contain a mitotic inhibitor, or chalone, which is not species-specific in its action.

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Survival after Fractionated Doses of Radiation: Modification by Anoxia of the Response of *Chlamydomonas*

IF ionizing radiation is delivered in fractions, cells usually recover from "sub-lethal damage" inflicted by one dose during the time which elapses before the next dose is given. This phenomenon has been observed with many types of cell, ranging from micro-organisms to higher