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Variations in the enzymatic behaviour of irradiated epidermis as function of age and lesion localization

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1. Introduction

Earlier reports have described variations of the epithelial response to high doses of X-rays delivered to the tail epidermis of new-born rats. There is a gradual tissue necrosis as a consequence of the changes produced by irradiation. Before ulceration starts, the epidermis shows variations, characterized by an acanthosis with considerable ultrastructural alterations and hyperkeratinization (de Rey and Cabrini, 1973, de Rey, Conti, Cabrini and Klein-Szanto 1976, Itoiz, Frasch, Volco and Klein-Szanto 1974, de Rey and Klein-Szanto 1972) as well as alterations in the content of SH and S–S groups (Frasch, Itoiz, Volco and Cabrini 1976) and of acid phosphatase (Itoiz *et al.* 1974), both indicating alterations in the production of horny substance.

There have also been repeated reports on glycolytic metabolism modifications, showing a constant decrease in enzyme activities related to the Krebs cycle, and an increase in those associated with the pentose shunt (Cabrini, Itoiz, Mayo, Smolko and Klein-Szanto 1970, Kelin-Szanto and Cabrini 1970, Klein-Szanto, Cabrini, Itoiz and Volco 1974). These enzymatic variations develop to a remarkable extent, as in the case of glucose-6-phosphatase dehydrogenase which, in some irradiation conditions, trebles the activity found in normal epithelia. Consequently, the activity of these enzymes could be used as possible detector of skin radiation damage. It therefore seemed interesting to study the influence of other factors, such as age or structure of the irradiated area, which might modify the degree of enzyme response.

2. Materials and methods

Ten Wistar rats, 3 and 12 days old, were irradiated on the right sole (a hairless area) and on the distal third portion of the tail. The rest of the body was protected with a lead shield. The left sole and the proximal two thirds of the tail were used as un-irradiated controls.

Irradiation conditions were: 230 kV, 13 mA, with filters of 0·25 mm Cu and 1 mm Al; with a dose-rate of 500 rad/min. Single total dose: 16 krad. The dosimetry was carried out simultaneously with a Simplex Universal Dosimeter with a low energy X-ray dosimetric chamber.

The animals were sacrificed 4 days after irradiation. Both soles and the tail of each animal were sectioned at the same time, without previous fixation, using a cryostat.

The sections were stained with the usual haematoxylin eosin (H–E)technique and with histochemical techniques for demonstration of glucose-6-phosphate

dehydrogenase (Gl.6.ph Dh), succinic dehydrogenase (SDh) and lactic dehydrogenase (LDh) (Nachlas, Tsou, De Souza, Chang and Seligman 1957, Hess, Scarpelli and Pearse 1958). Variations of the epithelial thickness were determined in the H–E stained sections by direct measurments with a micrometric eyepiece. The epidermal enzyme activities were determined with a microspectrophotometer provided with an automatic stage (Zeiss Cytoscan). The methodology of epidermal enzyme activity determinations has been described by Klein-Szanto and Cabrini (1970), Klein-Szanto *et al.* (1974) and Itoiz *et al.* (1974).

The total enzymatic content (TEC) of the epithelial layers, except that of the horny layer, was determined by a microspectrophotometric scann perpendicular to the basal membrane, i.e. in the basal-horny direction. The mean enzyme concentration (MEC) was determined by dividing the TEC by the epidermal thickness, which was equivalent to the length of the scanning. The MEC in fact represents the amount of enzyme activity per volume unit.

To compare and average the data of different animals, an experimental/control ratio was used. Each animal's control and irradiated epidermis were sectioned simultaneously, so that the use of the above mentioned ratio avoided possible errors introduced by section-thickness variations between different samples.

3. Results

3.1. *Epidermal thickness*

All animals, particularly the younger ones, showed abnormalities in the spinous and granular layers. Furthermore, the increase in thickness was larger in the tail than in the sole (figure 1).

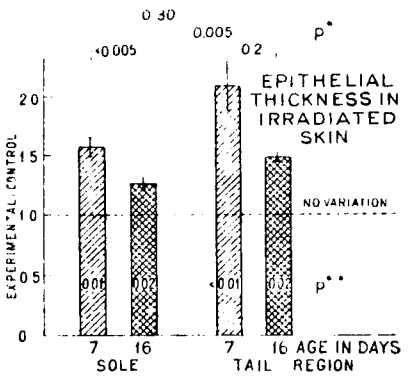

Figure 1. Epidermal thickness determined on H–E sections with a micrometric eye piece. Ordinates show the ratio R^E/C between experimental and control values. All animals were killed 4 days after irradiation. Bars indicate mean values from five animals ± 1 standard error. p** Probability, calculated with Student's test using paired experimental and control data of each animal. *P** = Probability, calculated with Student's test between the different regions and ages under study.

3.2. *Variations of the enzyme activity*

The total enzyme content (TEC) of SDh and LDh increased in all cases studied, except in the sole of 7-day-old animals. The LDh increases are smaller,

particularly for sole. The MEC values decrease, except the SDh in sole 16 days, indicating a smaller availability of both enzymes per unit volume. In all cases the most important variations occur in the tail and in the younger animals, a phenomenon coincident with the morphological alterations.

Gl.6.ph Dh showed a reactive increase in both TEC and MEC in all the conditions studied (figure 2). As with the other enzymes tested the largest variations were found in the younger animals. Although the total content of this enzyme is higher in the tail than in the sole, the weaker acanthotic reaction in the latter is responsible for the higher MEC values in that region.

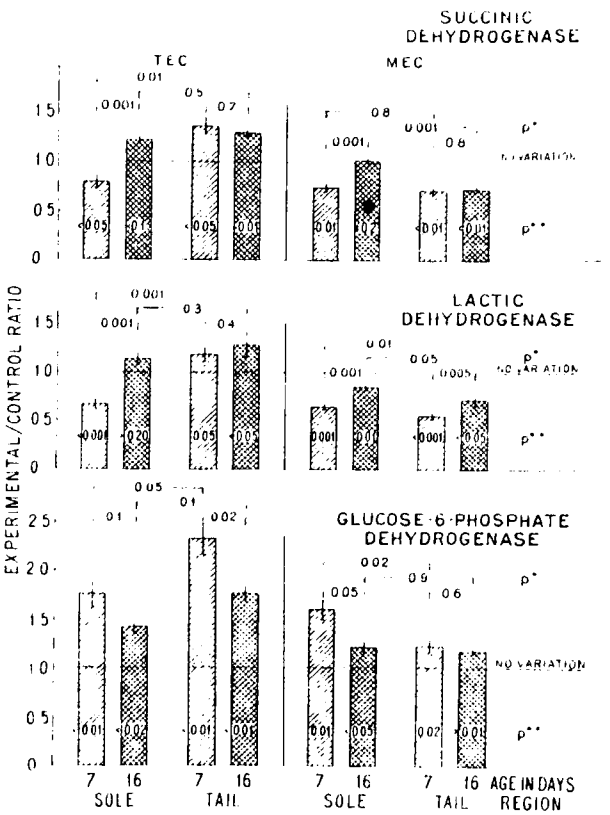

Figure 2. Total enzyme content (TEC) and mean enzyme concentration (MEC) of succinic dehydrogenase, lactic dehydrogenase and glucose-6-phosphate dehydrogenase, Ordinates show the ratio between irradiated and control values. All animals were killed 4 days after irradiation. Bars indicate mean values from five animals ± 1 standard error. p** = Probability, calculated with Student's test using paired experimental and control data of each animal. p* Probability, calculated with Student's test between the different regions and ages under study.

4. Discussion

These data confirmed previous results regarding the decrease of SDh and LDh and the increase in Gl.6.ph Dh, in the tail of 7-day-old animals (Klein-Szanto and Cabrini 1970, Klein-Szanto *et al.* 1974). The decrease in SDh indicates a reduction in the enzyme activities of the Krebs cycle. This phenomenon coincides with the decrease in volume fraction of mitochondria per

volume unit, demonstrated in epithelia irradiated with similar doses (de Rey *et al.* 1976).

On the other hand, the increased activity of the pentose shunt could produce a larger amount of intermediate products for the synthesis of RNA, which could in turn be used for the synthesis of proteins during keratinization (Bernstein, Chakrabarti and Kumaroo 1970). Consequently, before necrosis of the epithelium occurs, the acanthosis and hyperkeratosis usually associated with enzyme changes can be understood as an abortive defensive response of the epidermis or as a speeding-up of the differentiation and cellular death mechanisms normally taking place in squamous epithelia.

The present findings show that young animals suffer larger morphological and enzyme alterations of the epidermis when exposed to radiation. The alterations vary according to the region studied. However, even though the acanthosis is not very pronounced, as for example on the sole, the response of the enzymes is significantly different from that of the controls.

The differences observed between the sole and the tail seem to indicate that the presence of hair follicles in the tail increases the degree of damage produced. It has been demonstrated that the enzyme activity of normal epidermis undergoes fluctuations, depending on the hair's growth-cycle (Argyris 1976). Hair follicles are very radiosensitive and the damage caused to them by radiation is probably reflected directly or indirectly in the metabolic activity of the epidermis.

Another phenomenon deserving attention is that vascularization differences between the sole and the tail can also influence the intensity of the epidermical response to radiation. The whole circulation of the tail depends on a large central artery and two small lateral branches which, if damaged early by radiation, impair the entire blood supply of the subepidermis region. In the sole, this alteration develops slowly because there is a far more complex blood-vessel net (Greene 1968). It is therefore necessary to establish clearly the degree of interrelation between the epidermical responses and the subjacent vascular alterations. At present, studies are being carried out to evaluate this interrelation.

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