THE EFFECT OF X-IRRADIATION COMPARED TO AN APPARENTLY SPECIFIC EARLY EFFECT OF SKIN CARCINOGENS†

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An early test for possible skin carcinogens has recently been described by Iversen (1961). He found good correlation between carcinogenic activity and the type of reaction that was recorded after one day by means of a tetrazolium reduction method (Iversen, 1959). In a blind test of twenty different compounds, the method singled out six carcinogens (e.g. 1.2,5.6-dibenzanthracene, 3.4-benzpyrene, and others) from the fourteen chemically-related but non-carcinogenic compounds. With the carcinogenic potentialities of X-irradiation in mind, it is of interest to compare the effect of X-irradiation with that of chemical carcinogens recorded by this method.

The tetrazolium method as applied by Iversen was used to estimate changes in the rate of formazan deposition in the epidermis of hairless mice, following application of different skin irritants. The skin was taken immediately after sacrificing the mice, and incubated for one hour at 37°C in a tetrazolium solution, with no substrate added. The reduction of tetrazolium salts by living cells is considered to be an indicator of dehydrogenase activities of the cells. The formazan formed is insoluble and is deposited in the cells at the site of reduction. The formazan is coloured, and the amount in the epidermis is measured by colorimetric methods.

The carcinogenic compounds produced an initial rise in formazan deposition on the first day, whereas the non-carcinogenic compounds showed a more or less pronounced depression at the same time (Fig. 1). Iversen has discussed the significance of the test, and considers that the increased deposition of formazan signifies a disturbance in the function of the mitochondria, with release of enzymes (Iversen and Evensen, 1962).

In collaborative work with Iversen, the effect of local X-irradiation on the epidermis of the same strain of mice has been tested in the same way as for the carcinogens, using X-ray doses ranging from 500 to 2,700 r (Iversen and Devik, 1962). A gradual increase in formazan deposition was observed, which was more pronounced the higher the dose, but more delayed in time when

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following the application of the carcinogens (Fig. 2). On the other hand, there was no clear indication of any initial decrease, as with the non-carcinogenic compounds.

One main difference between X-radiation and toxic or pharmaceutical substances is the distribution in space: the former acts very uniformly at the

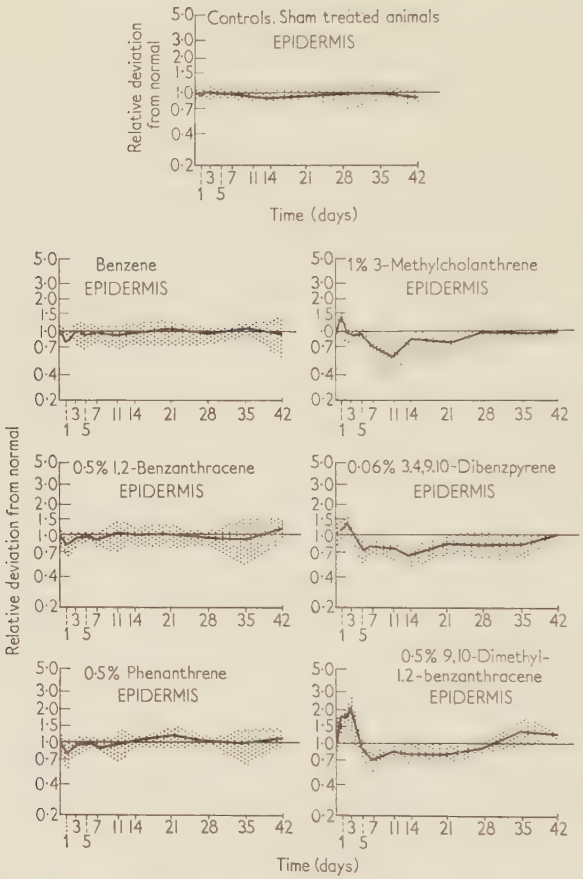

Fig. 1. Formazan deposition in the epidermis after application of three non-carcinogenic compounds (to the left), and three carcinogenic compounds (to the right), relative to the deposition in normal epidermis. Reproduced by kind permission of Dr. O. H. Iversen (Iversen and Evensen, 1962).

cellular level, whereas the latter are expected to show differences in concentration both at the cellular, and the subcellular, level. For this reason one should not expect too close a parallelism neither with respect to the acute nor to the late reactions and effects.

Without bringing out any similarity which is immediately striking, the experiments do indicate that a functional disturbance of mitochondria may be common to chemical carcinogens and to X-irradiation. Whether the disturbance is closely related to the mechanism of carcinogenesis remains to be determined.

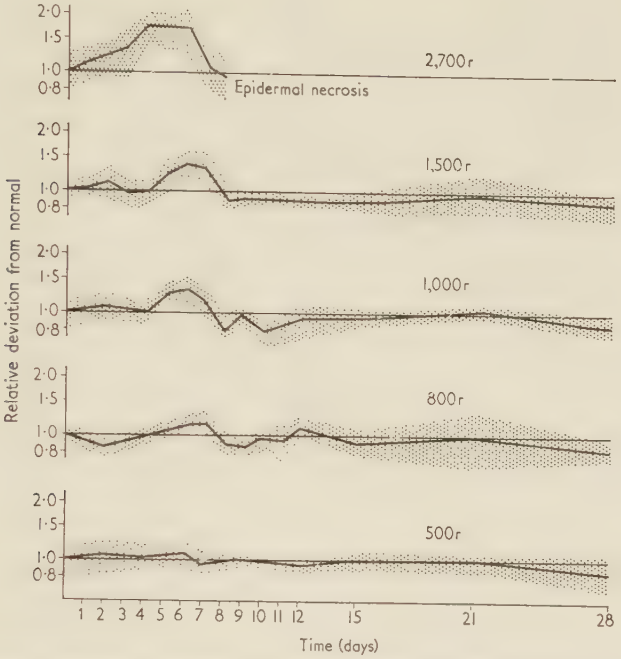

Fig. 2. Relative amounts of formazan deposited in epidermis after irradiation. (Reproduced by kind permission of the Editor of *International Journal of Radiation Biology*.)

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DISCUSSION

Glücksmann: May I ask one question? In both cases you would get hyperkeratosis in the skin, from the carcinogens as well as from the radiation injury. Does your reaction simply mean that you have different types of cells present in the skin?
Devik: This I do not know, but this reaction is recorded very early, well before you get any keratosis. We can hardly differentiate histologically in our experiments between irradiated and non-irradiated skin at an early age.

BACQ: I wish to point out to Dr. Devik that Tabashnik from the U.S.A. has recently published a paper showing the liberation of RNase in the skin very early after irradiation of guinea-pigs with β-rays of ^{90}Sr. He points out that his test is very good because he has no changing population, he has no dead cells and no reproduction, so that he is constantly dealing with the same population of cells.

UPTON: May I ask, how sharply localized the change was? Did you find any diffusion of activity along the margin of the irradiated area?

DEVIK: The irradiation was very sharply localized. We only sampled the epidermis well within the irradiated field, we did not examine the margin.

UPTON: Due to the finding that tumour formation tends to occur at the edges, it would be be interesting to find out how sharply localized this change might be in relation to the margin of the irradiated area.

DEVIK: We have excluded so far the periphery of the field.

KOLLER: I would like to mention our experiments with skin autografts after total-body irradiation. In a control experiment we also transferred skin from an irradiated animal to these mice. And we were very impressed that within twenty-four hours we had such a thickening of the skin after irradiation. The effect is very dramatic and very quick.

DEVIK: What dose did you use?

KOLLER: The LD_{99}.