

THE ACTION OF X-RAY IRRADIATION AND PAINTING WITH CARCINOGENIC HYDROCARBONS ON THE PHOSPHORUS CONTENT OF THE EPIDERMIS*

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The present investigations deal with the question of whether contact X-ray irradiation as well as painting with methylcholanthrene and benzpyrene effect a change in the total phosphorus content of the epidermis of the treated area of the skin.

METHOD

The X-ray experiments were carried out on white male and female rats. One group of animals weighed 60 g, and the other group weighed 100–120 g. The hair was plucked on both the sides of the animals in round symmetrical areas, the diameters of which were about 2.5 cm. On one of the areas X-ray irradiation was applied, the opposite side was used as control. The X-ray irradiation was done 9–11 days after the hair had been plucked. At this time, in the majority of the animals, the new hairs had not yet reached the surface of the skin, or were only just perceptible. (The animals in which the hairs were already a few mm long on the 9–11 day after the plucking were not used for the experiment.) The plucking was done so that the hair on the area of the skin of the animals used for the experiment would be approximately at the same stage of development since our preliminary experiments indicated that the reaction of the irradiated skin depends to a great extent upon the stage of the development of the hair. Each animal received a dose of 3000 r contact X-rays in one field. The irradiation was carried out by means of a Philips contact apparatus (2 mA, 50 kV and a focal distance of 20 cm) without using a filter. The examinations were carried out on the 2–7th day after the irradiation. At this time the hairs on the areas of the skin treated with X-rays, as well as on that of the controls, were already long enough to be plucked out with pincers. The loosening of the hairs on the sites treated with X-rays could be detected 2–3 days after the irradiation.

The investigations concerning the action exerted by the carcinogenous hydrocarbons were carried out on domestically bred, light brown colored adult mice. Most of the animals were males, only a few of them females. The hairs of the animals were—at least apparently—in the resting stage. Animals showing signs that the hairs were in a stage of changing were not used. The skin of one side of the animals was painted on a round area 2 cm in diameter with a solution of 0.6 per cent of methylcholanthrene or 0.8 per cent of benzpyrene dissolved in benzene, without the hair being sheared. Most of the animals were painted only once, in a few cases twice. The second painting, when done, was carried out on the 4th day after the first. The examinations were performed on the 1–6th day

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after the first painting and on the first and second day after the second one. As control a symmetrical site of the skin of the opposite side of the animals was used.

When the observations were concluded, the animals were sacrificed and the hairs plucked from the treated sites (X-rays or painting) as well as from the control skin. After the excision of the skin the remaining hairs were very carefully removed. This was carried out with much care because the large quantity of nitrogen and low quantity of phosphorus contained in the keratin interferes to a great extent with the exactness of the results. Subsequently, the surface of the skin areas was wiped repeatedly with cotton wool moistened with distilled water and the epidermis was then separated from the portion of the connective tissue. The separation was performed with the method described in a previous paper (1), the only difference being that instead of using an ammonium hydroxide solution one of potassium hydroxide was employed. With the latter solution the separation was far more rapid, lasting only 2-3 minutes. Subsequently the epidermis was incinerated with concentrated sulphuric acid and perhydrol and its nitrogen and phosphorus content determined colorimetrically. The phosphorus determination was carried out according to Horwitz's (2) method; for the nitrogen determination, Nessler's reagent was employed. The results were recorded by means of a Pulfrich-photometer. The phosphorus content was related to the total nitrogen content.

RESULTS

Irradiation with 3000 r contact X-rays resulted in a considerable decrease of the total phosphorus content of the epidermis of each of the animals used for the experiment. In the animals weighing 60 g, the diminution could be detected on the 3rd day after the irradiation, whereas in the epidermis of those weighing 100-120 g it was first observed on the 4th day. The extent of the decrease was almost the same from the 3rd or 4th day on, until the 7th day. The results of the X-ray experiments are illustrated on Table I.

On the first day after the first painting with a methyleholanthrene solution of

TABLE I

The change in the phosphorus content of the epidermis following irradiation with 3000 r contact X-rays in rats

Beside the average values, the standard deviation is given

Time After Irradiation	The Numbers of the Examinations on Rats Weighing 60 g	Irradiated Control		The Numbers of the Examinations on Rats Weighing 120 g	Irradiated Control	
		P _γ /100 _γ N	P _γ /100 _γ N		P _γ /100 _γ N	P _γ /100 _γ N
2 day	5	8.3 ± 0.9	8.1 ± 0.9			
3 day	9	5.0 ± 0.7	8.0 ± 0.8	6	7.5 ± 0.4	7.6 ± 0.9
4 day	14	4.7 ± 0.7	7.8 ± 1.2	6	5.6 ± 0.5	7.9 ± 0.8
5 day				7	5.6 ± 0.9	8.2 ± 1.5
7 day				7	5.0 ± 0.5	8.3 ± 1.1

0.6 per cent, the phosphorus content of the epidermis was completely identical with that of the control one. In 30 mice examined on the second day after the painting, an appreciable decrease could be observed in the phosphorus content of the epidermis in 25 of the 30 animals. In 5 animals, the amount of phosphorus contained in the painted epidermis was identical with that of the control. On the 3rd day after the application, the phosphorus content of the epidermis of all of the painted animals showed a significant decrease. However, the decrease was slightly less than that observed on the second day. On the 4th and 6th day following the painting, the phosphorus content of the treated epidermis was nearly the same as that of the normal one. In view of the fact that after a treatment with a benzpyrene solution of 0.8 per cent essentially identical results were obtained, these data are not reported in detail. In the animals painted with methylcholanthrene in which on the 4th day after the treatment the painting was repeated on the same area of the epidermis, the decrease on the first day following the second painting was more appreciable than in the other cases. On the second day after the second painting the phosphorus content again approached the normal values. It should be mentioned that on the second day after the second painting the treated sites were covered with a dry, rather toughly adhering epidermis under which fresh epidermis could be detected. The examinations concern the latter.

The benzene used as a solvent did not—according to the examinations carried out on 35 animals—influence the phosphorus content of the epidermis. The investigations relating to the carcinogenous hydrocarbons are summarized on Table II.

TABLE II

The change in the phosphorus content of the epidermis after methylcholanthrene and benzpyrene painting in mice

Beside the average values, the standard deviation is given

Time After First Painting	Methylcholanthrene			Benzpyrene		
	The numbers of the ex-aminations	Painted	Control	The numbers of the ex-aminations	Painted	Control
		P _γ /100 _γ N	P _γ /100 _γ N		P _γ /100 _γ N	P _γ /100 _γ N
1 day	9	10.7 ± 1.5	10.5 ± 1.1	8	10.4 ± 1.4	10.4 ± 1.4
2 day	5	10.2 ± 0.4	10.2 ± 1.5	5	10.2 ± 1.3	10.6 ± 1.3
2 day	25	6.9 ± 1.0	10.3 ± 1.5	20	7.1 ± 1.0	10.2 ± 1.2
3 day	19	7.5 ± 1.0	10.4 ± 1.2	24	7.4 ± 1.3	10.3 ± 1.4
4 day	10	9 ± 1.4	10.1 ± 1.4	10	9.3 ± 1.5	9.9 ± 1.2
5 day	11	9.2 ± 1.4	9.9 ± 1.4	10	9.1 ± 1.4	10.3 ± 1.1
6 day	10	9.5 ± 1.0	10.1 ± 1.5	8	9.6 ± 1.0	10.4 ± 1.5
After Second Painting						
1 day	8	5.6 ± 1.0	10.2 ± 1.4			
2 day	7	9.5 ± 1.2	10.5 ± 1.4			

DISCUSSION

The decrease of the total phosphorus content of the epidermis following X-ray irradiation and painting with methylcholanthrene and benzpyrene must produce a significant change in the life of the cell involving the phosphorus compounds and probably the whole phosphorus metabolism. Further detailed investigations should be carried out.

The literature available to us contains a few data which can be brought into correlation with our experimental results. Kausche (3) found that the phosphorus content of a virus strain which underwent mutation on the action of X-rays had decreased. Following total X-ray irradiation with 600 r, Lutwak-Mann (4) observed a diminishment of the desoxyribonucleic acid in the bone marrow and spleen of rat. Thomson, Tourtelotte and Carttal (5) noted a pronounced change in the thymus gland of rats due to total irradiation with gamma rays. They observed an appreciable decrease in the phosphorus content and in the P^{32} uptake of desoxyribonucleic acid and of ribonucleic acid, as well as a reduction of the capacity of the thymus homogenate to esterify inorganic phosphate. Thomson and co-workers (6) noted in rabbits, in the bone marrow of one of the limbs irradiated with gamma rays, a decrease of the desoxyribonucleid phosphorus acid- and ribonucleic acid content after treatment. Costello and co-workers (7) detected a decrease of the phospholipid content of the epidermis resulting from painting with methylcholanthrene.

In addition to the effects mentioned above, thallium, which exerts in many respects a radiomimetic action, but is not according to present knowledge carcinogenic, also impairs the phosphorus metabolism. This is supported by the following data: Fiacco (8) and Fanton (9) observed phosphaturia in experimental animals exposed to thallium action. According to Rominger, Meyer and Bomskov (10) the primary cause of the bone damage due to thallium is the diminished P_2O_5 retention. Rygh (11) believes that the decalcification effected by thallium starts with the removal of the phosphate ions. Our own investigations (12) demonstrate that the surface of the hair bulbs epilated on the action of both thallium and X-rays has no inorganic phosphate layer like the healthy hair bulbs have. Of course, these data relating to thallium do not render less important the investigation of the changes taking place in the phosphorus metabolism in the course of carcinogenesis.

SUMMARY

In rats, contact X-ray irradiation with 3000 r and in mice one painting with methylcholanthrene and benzpyrene resulted in a pronounced decrease of the total phosphorus content of the epidermis. Following X-ray treatment, the extent of the decrease was greater and of longer duration, whereas in the case of chemical carcinogens, the phosphorus content returned almost to normal on the 4th day after the treatment. Repeated painting with methylcholanthrene again effected a diminution of the phosphorus content of the epidermis which was even more pronounced than that following the first application.

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