THE EFFECT OF X-RAYS ON THE PHOSPHORUS CONTENT OF THE DESOXYRIBONUCLEIC ACID, RIBONUCLEIC ACID AND LIPOID FRACTIONS OF THE EPIDERMIS IN RATS*

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The clinical effect of X-rays, as well as some of their effects on the morphology of certain tissues, is well known. Their biochemical effects, however, have not been studied extensively. Thomson and co-workers (1) have shown that in rats the desoxyribonucleic acid (DNA) and to a smaller extent the ribonucleic acid (RNA) content of the thymus decreases on exposure to 800 R of gamma rays. It has also been established (2) that following irradiation of 310–620 R of gamma rays, the concentration of RNA and that of DNA in the bone marrow of rabbits decreases markedly. Lutwak-Mann (3) observed the decrease of the phosphorus content of the nucleic acid in the bone marrow of rats. Holló and Zlatarov (4) noted a pronounced diminution of the total phosphorus content of the epidermis of rats following contact X-ray irradiation.

Investigators have examined the changes due to irradiation with contact X-rays in the phosphorus content of the DNA, RNA and lipoid fractions of the epidermis. The hair of white rats, of both sexes, weighing 80–120 gm. was plucked on both sides of the animals in an area of 3 x 6 cm. On the 9th to 11th day following the plucking, a dose of 3000 R was applied to one side, in two fields, with a Philips contact apparatus (2 mA, 50 kV and a focal distance of 20 mm) without filtration. The epidermis of the other side of the animal was used as control. The plucking was carried out prior to the start of the experiment in order that the hair on the areas used for the experiment would be approximately at the same stage of development. At the time of the X-ray irradiation the new hairs had not yet reached the surface of the skin, or were only barely visible. The animals in which the hairs were a few mm. long were not used for the experiment. Observations were made on the 2nd, 3rd, 4th, 5th and 7th day. By this time the hair on the areas of the skin treated with X-rays, as well as on that of the controls, were long enough to be plucked out with pincers. On the day of the examination the animals were sacrificed and the hairs on the areas of the skin used for the experiment removed. After the 3rd day following irradiation, the removal of the hairs became progressively easier. From the 5th day on, the epidermis, which was swollen because of irradiation, could be easily injured and had to be treated with great care. The epidermis was separated from the connective tissue by means of a N solution of potassium hydroxide (KOH) using the method of Holló and Zlatarov (4, 5).

The analysis of the substance obtained in this manner was carried out by combining the methods described by Schmidt and Thannhauser (6) and Schneider (7), and as described by Holló and Zlatarov (8) in their work with the mouse.

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epidermis. The examinations were carried out on the 2nd, on the 3rd and on the 4th day on 18 animals respectively; on the 5th day on 24 animals and on the 7th day on 12 animals. For one experiment the irradiated and control areas of the epidermis of 6 animals were collected. Whereas with KOH the separation of the epidermis on the irradiated areas could always be performed rapidly and smoothly, the control areas occasionally presented discrepancies. In several experiments, the results obtained showed lower values in the control material than in the normal ones usually. The histological control experiments showed that in these cases the separation of the epidermis was not complete, and 20–30 per cent of the stratum basalis had not been separated from the papillae. These results were of course not taken into consideration.

The results are summarized in table I and show that the phosphorus content of the lipoid, RNA and DNA fractions of the epidermis undergoes a significant change.

The decrease in the phosphorus content of the lipoid fraction could be observed as early as the second day and did not show an increasing tendency even on the 7th day.

The phosphorus content of the RNA gave normal values on the 2nd day, but decreased gradually from the 3rd to the 7th day.

The phosphorus content of the DNA exhibits the most pronounced alteration. On the 2nd day a considerable diminution was observed, and on the 5th day was in excess of 50 per cent.

On comparing the control values with the phospho-proteins of the epidermis, the latter did not exhibit any change (6–7 gamma P/100 mg. fresh epidermis).

In several cases the nitrogen content of the DNA fraction was also determined. It is interesting to note that in the case of appreciably diminished phosphorus values, the amount of nitrogen contained in the irradiated epidermis was almost identical to that found in the controls. Calculated for 100 mg. fresh weight, the average of nitrogen weight contained in the DNA fraction was 380 gamma, whereas that of the irradiated epidermis was 370.

<table>
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<th>Time of Examination</th>
<th>Number of Examinations</th>
<th>X-rays treated</th>
<th>Controls</th>
<th>X-rays treated</th>
<th>Controls</th>
<th>X-rays treated</th>
<th>Controls</th>
</tr>
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<tr>
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<td>32.7 ± 7.3</td>
<td>15.1 ± 5.0</td>
<td>54.4 ± 2.0</td>
<td>40.3 ± 6.2</td>
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</tr>
<tr>
<td>3</td>
<td>3</td>
<td>26.0 ± 4.5</td>
<td>13.8 ± 1.2</td>
<td>43.3 ± 3.3</td>
<td>35.9 ± 6.2</td>
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</tr>
<tr>
<td>4</td>
<td>3</td>
<td>24.7 ± 1.7</td>
<td>13.2 ± 6.1</td>
<td>43.3 ± 3.3</td>
<td>35.9 ± 6.2</td>
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<tr>
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<tr>
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</table>

The amount of phosphorus contained in the DNA-, RNA- and lipoid-fractions of the X-ray treated and control epidermis. Besides the mean values, the average deviation is demonstrated. Values: γ phosphorus/100 mg. fresh epidermis. For one experiment the irradiated and control areas of the epidermis of 6 animals were collected.
Thus it has been shown that on the action of irradiation with contact X-rays, the phosphorus content of DNA shows a marked decrease before the swelling of the epidermis and the epidermolysis sets in. The same obtains, only to a lesser extent, for that of the phosphorus of the lipoid fraction. From the 3rd day on, the phosphorus content of the RNA fraction also decreases, and the decrease of the two former substances becomes consistently more pronounced. Considering that these significant changes coincide with the loosening of the hair, the swelling of the epidermis and the epidermolysis, it seems probable that there is a correlation between the morphological and biological changes.

The resemblance between the results we obtained after irradiation with X-rays and those of Holló and Zlatarov (8) resulting from painting with methylcholanthrene is striking. On applying a single painting with a solution of 6 per cent of methylcholanthrene, the phosphorus content of the DNA fraction of the epidermis decreased considerably, whereas its nitrogen content remained unchanged. Painting with methylcholanthrene also results in a significant decrease of the phosphorus content of the lipoid fraction of the epidermis. The only difference which could be observed is the behavior of the phosphorus content of the RNA fraction, inasmuch as after showing a transitory decrease, it exceeds on the 4th day the normal values, whereas on the action of X-rays it decreases gradually until the 7th day. These data may indicate that different carcinogenous agents, such as chemical agents and X-rays, lead by means of identical biochemical alterations to the development of malignant cells.

SUMMARY

On the action of contact X-ray irradiation (3000 R) the following changes could be observed in the epidermis of rats: from the second day on after the irradiation, the phosphorus content of the lipoid and DNA fractions decreased and after the 3rd day that of the RNA showed the same phenomenon. The diminution of these values continued uninterruptedly until the 7th day. It was the most pronounced in the case of the DNA fraction which showed a change exceeding 50 per cent on the 7th day. The phosphorus values of the protein fractions did not exhibit any alteration on the action of contact X-rays. The nitrogen content of the DNA fraction remained unchanged in spite of the decrease of the phosphorus values.

REFERENCES
5. ZLATAROV, S. AND M. HOLLÓ: Die Wirkung der Röntgenbestrahlung und des Thalliums

