Effects of X-ray Radiation on HeLa Cells

KANNO, Iwao*, FUKUSHI, Kazue*, YAMAGUCHI, Junji*, UTAGAWA, Keiji*,
UMEHARA, Eiju*, SATO, Kazuo* and Yasuhiko ITO*

(Received Nov. 29, 1965)

ABSTRACT

Observations were made from various points on the effect of single X-ray irradiation at 100 to 1,000 R on HeLa cells: 1) The metabolism was disturbed in HeLa cells in culture in test tubes by X-irradiation at more than 300 R. With over 300 R, the proliferation of the number of cell nuclei was restrained. This effect was especially pronounced with irradiation by 1,000 R. 2) Examination by Giemsa staining revealed only the disturbance in mitotic cells in X-irradiation at less than 200 R, but the disturbance in the resting cells was pronounced in X-irradiation at over 500 R. 3) Histochemical observation by means of Feulgen reaction revealed that more than 20% of the stickiness of chromosome was produced in 3 to 7 days after X-irradiation by 500 to 1,000 R. 4) Observation of electron-microscopic pictures revealed that the cytoplasm of HeLa cells grew larger with the increase of the amount of X-irradiation and that round degenerations were present locally. Both cytoplasm and nucleoplasm became scarce. No remarkable changes were observed in mitochondria, endoplasmic reticulum and nucleolus. The double structure of the nuclear membrane remained undisturbed. 5) The inhibitions of about 18% in the respiratory action and 29% in glycolysis were observed 24 hours after X-irradiation at 1,000 R. 6) There was hardly any change in the amount of nucleic acid (both RNA-P and DNA-P) 15 hours after X-irradiation at 1,000 R, but the incorporation of H3P into nucleic acid-P was clearly inhibited. Also about 50% inhibition of the mononucleotide of the RNA was observed. No remarkable difference in incorporation was observed among the mononucleotides in the groups.

* The Research Institute for Tuberculosis and Leprosy, Tohoku University, Sendai, Japan
INTRODUCTION

Studies have been made from various points of view on the effect of radiation on malignant tumors by many researchers. It was observed in 1924 by Alberti et al. that 10 to 40 minutes' X-irradiation resulted in a rapid decrease in the number of mitotic cell divisions in the epithelial cells of the cornea amphibian larvae. They called it the primary effect of X-irradiation. The mitotic cell divisions completely disappeared 10 hours after X-irradiation. This was called the secondary effect of X-irradiation. They reported that the former was due to a disturbance of the chromatin and that was characterized by contraction of the cells and the latter by the disturbance of the polar movement of the chromosome. The continuation of the primary effect was independent of the irradiated dose. This study is of use to us in getting information on the effect of X-ray on the cells. We have very few reports, however, on the effect of X-irradiation from various angles within the level of the tumor cells. In the following, a report is made of the morphological, histochemical, electron-microscopic and biochemical effects of X-irradiation in HeLa cells.

EXPERIMENTAL METHOD

1. Method of the culture of HeLa cells

As is well known, HeLa cells are thought to be the epithelial cancer-cells cultured histologically from cervical cancer of the human uterus. Reports have been made on the culture of HeLa cells by Gey et al., Evano et al. and Rinaldini et al. In the present experiments, HeLa cells 5 days after culture were treated in 0.25 trypsin (Difco 1:250)-Rinaldini solution and Rinaldini solution, suspended in 20% cow serum plus YLE medium (NaCl 7.18 g, KCl 0.4 g, CaCl₂ 0.2 g, MgSO₄·7 H₂O 2 g, NaH₂PO₄·2 H₂O 0.16 g, Dextrose 4.5 g, NaHCO₃ 1.1 g, Lactalbumin hydrolysate 5.0 g, Eastextract 1.0 g, phenol red solution 1.5 ml, streptomycin 200 μg/ml, penicillin 100 u./ml, distilled water 700 ml) and allowed to stand at 37°C under the conditions to meet various purposes of the experiments. (a) HeLa cells used for calculating the number of cell nuclei: 50,000/ml in suspension was poured, 2 ml each, into test tubes which were inclined 5°C for culture. (b) HeLa cells used for Giemsa staining and for Feulgen reaction: 50,000/ml in suspension was poured, 2 ml each, into D-3.5 Carrel's bottles with over-glass pieces inserted in them for culture. (c) HeLa cells used for electron-microscopic observation: 200,000/ml in suspension was poured, 50 ml each, into 500 ml Roux's bottles for culture. (d) HeLa cells used for determining the respiration and glycolysis of HeLa cells: 400,000/ml in suspension was poured, 4 ml each, into Warburg's flasks (40 ml) for culture. (e) HeLa cells used for measuring metabolism of nucleic acid: 200,000/ml in suspension was poured, 50 ml each, into 500 ml Roux's bottles for culture.

In each of the cases mentioned above, the media were changed on the third day of cultivation and they were incubated for several hours at 37°C. Then they
were subjected to X-irradiation by the method described in the following, and the media were exchanged every second day to maintain culture.

2. Irradiation method

(1) Conditions for irradiation: Toshiba's KXC-18-II type apparatus for the deep therapy was used. The conditions for irradiation were the following. Tube electric pressure: 180 KVp; Tube electric current: 3 mA; Filter: Cu 0.7 mm + Al 0.5 mm; FSD (Focus-Subject Distance): 40 cm; The field size 25×25 cm²; Air dose: 10.26 r/min. (2) X-ray was irradiated directly on the outer surface of the tubes in which HeLa cells were cultured, at 100 R, 200 R, 300 R, 500 R and 1000 R of air doses respectively.

3. Observations

(1) Measurement of the number of HeLa cell nuclei: The following method was adapted in calculating the number of cell nuclei. The test tubes just before the X-ray irradiation (for control) and those 3 days and 7 days after X-irradiation (with doses of 100 R, 200 R, 300 R, 500 R and 1000 R respectively) were taken out and centrifuged at 1,500 r.p.m. for 15 minutes before beginning culture. 5 ml of cytric acid solution (21 g of cytric acid, 500 mg of Crystalviolet, 10 drops of formalin and 1,000 ml of distilled water were added to 0.5 ml of the medium remaining in the tube after removing the supernatant liquid. It was then allowed to stand in an incubator at 37°C for several hours with shaking at intervals. Then it was agitated with a pipett, transferred to a small test tube graduated accurately at 1 ml and centrifuged at 1,500 r.p.m. for 15 minutes. The supernatant liquid was then removed, leaving 1 ml, and agitated. A drop of it was placed on the Bürker's counting chamber and the number of nuclei were counted. (2) Observation by Giemsa staining: Using HeLa cells irradiated with 100 R, 200 R, 300 R, 500 R and 1,000 R of X-rays, the coverglass pieces were taken out of the Carrel's bottles at 5, 12 and 24 hours and 3, 5 and 7 days after the X-irradiation, solidified in Carnoy solution, Giemsa stained and examined microscopically in the sealed balsam. (3) Observation of changes in HeLa cell nucleus by means of the Feulgen reaction: After irradiating HeLa cells with 200 R, 300 R, 500 R and 1,000 R of X-rays, they were solidified in the Carnoy solution at 5, 12 and 24 hours and 2, 5 and 7 days after X-irradiation and subjected to the Feulgen reaction to observe changes in the cell nucleus. (4) Electron microscopic observation: Examination was made at 12 hours and 3 days after irradiation of HeLa cells radiated with 200 R, 300 R and 1,000 R of X-rays. The specimens were prepared by adding a small quantity of 1% phosphate buffered osmic solution (pH 7.4) for preliminary solidification. The cells fixed on the wall of the vessel were exfoliated with a sponge, and then centrifuged at 1,000 r.p.m. for 5 minutes. The supernatant liquid was removed and the solidified solution mentioned above was added to the cell sediment and solidified at room temperature for 20 minutes. They were then dehydrated by 50, 70, 90 and 100% ethanol series and enveloped with methacrylate. After finishing polymerization, ultra-thin slices were prepared by using LKB Sjöstrand ultra microtom and micro-
scopic photographs were taken of them by HU-10 type electronmicroscope (accelerated voltage 75 KV) of the Hitachi & Co. (5) Observation by means of the metabolism of respiration and glycolysis: Examination was made at 5 and 24 hours concerning the effect of radiation with 1,000 R of X-rays on respiration and glycolysis. For measuring respiration, HeLa cells were suspended in mixture with Krebs-Ringer phosphate buffer solution, and for measuring glycolysis, HeLa cells were suspended in mixture with Krebs-Ringer-bicarbonate buffer solution. Respiration per 1,000,000 cells and anaerobic glycolysis were measured from the consumed quantity of oxygen and the generated quantity of carbon dioxide by the standard manometric technique of Warburg. (6) Observation through metabolism of nucleic acid: With HeLa cells irradiated with 1,000 R of X-rays (single irradiation), observation was made on the changes in nucleic acid at 3 and 15 hours after the X-irradiation. That is, studies were made on the quantitative analysis of nucleic acid, incorporation of $^{32}$P into nucleic acid-P and the effect of RNA on mononucleotide.

RESULTS OF EXPERIMENT

1. Effect of X-irradiation on the metabolism of HeLa cells

The media were adjusted to pH 7.6-7.2, and the test tubes containing HeLa cells in culture and those without HeLa cells were stained by pouring in a phenolred solution (0.1 g of phenolred, 3.0 ml of ethanol and 7.0 ml of distilled water). Generally, when metabolism occurs in HeLa cells, the solution in the test tube changed yellow and the color tone grew intense proportionally to the intensity of metabolism. Let us compare a non-X-irradiation group (control), the X-irradiation groups (with 100 R, 200 R, 300 R, 500 R and 1,000 R respectively) and a group without HeLa cells. The non-X-irradiation group containing HeLa cells turned yellow as soon as phenolred was added. The group without HeLa cells turned red with a color tone of phenol-red. The X-irradiation groups showed various colors both light yellow and many shades between yellow and red. That is, a slightly reddish color could be observed in the groups radiated with over 300 R X-ray. The group irradiated with 1,000 R X-rays showed a somewhat yellowish color, though red in color tone close to that of the group without HeLa cells. This clearly shows that X-irradiation disturbs the metabolism of HeLa cells. According to the report made by Dawson et al.\(^b\), the HeLa cells were still living when they were irradiated at 80, 160 and 240 R of X-irradiation after being frozen for 1 hour. With freezing alone, the rate of survival was 67%, and with the addition of 10% dimethylsulphoxide, the rate of survival did not lower at all.

2. Variation in the number of HeLa cell nuclei

As seen in Fig. 1, the cells increased in number with the passage of culture time in the non-irradiated group. The observation of the effect of radiation at the third day of culture clearly showed that the irradiation at more than 300 R would restrain the increase in the number of cells. This was more pronounced in the
case of irradiation at 1,000 R. According to the report by Kaplan et al.\(^9\), irradiation with a small quantity of X-rays inhibited mitosis temporarily, moderate irradiation caused disturbance in chromosome and large irradiation made the division of cells impossible. It also states that the formation of DNA continued regardless of the inhibition of the mitotic division of the cells.

3. Observation by means of Giemsa staining

As seen in Table 1, in the irradiation at less than 200 R, only the mitotic

Table 1. Observation by means of Giemsa staining of HeLa cell after X-irradiation

<table>
<thead>
<tr>
<th>Doses of X-ray</th>
<th>5 hrs.</th>
<th>12 hrs.</th>
<th>24 hrs.</th>
<th>3 days</th>
<th>5 days</th>
<th>7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 R</td>
<td>○</td>
<td>○ + ○</td>
<td>○ + △</td>
<td>△</td>
<td>△</td>
<td>△</td>
</tr>
<tr>
<td>200 R</td>
<td>○</td>
<td>○ + ○</td>
<td>○ + △</td>
<td>△</td>
<td>△</td>
<td>△</td>
</tr>
<tr>
<td>300 R</td>
<td>○</td>
<td>○</td>
<td>○ + △</td>
<td>△</td>
<td>△</td>
<td>△</td>
</tr>
<tr>
<td>500 R</td>
<td>○</td>
<td>○</td>
<td>○ + △</td>
<td>△</td>
<td>△</td>
<td>△</td>
</tr>
<tr>
<td>1,000 R</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>△</td>
<td>△</td>
<td>△</td>
</tr>
</tbody>
</table>

Remark: ○ breakage of mitotic cell  ▲ cell division (+)
△ similar to control        ● disturbance of resting cell (±)
○ recovery of mitotic division  ○ disturbance of resting cell (+)
division disturbance could be observed. The disturbance of not only mitosis but also of the resting cells was remarkable on the third day of irradiation at more than 500 R. Plate 1a shows HeLa cells in the non-irradiated group on the 4th day. Pro-, Meta-, Ana-, Telo-phases can also be seen. In Plate 1b, 12 hours after the X-irradiation with 100 R, the breakage of mitosis can be seen. In Plate 1c, three days

1 a Control
4 days after culture HeLa cells in the non-radiation at various phases can be seen.

1 b 12 hours after X-irradiation at 100 R.
The breakage of mitosis can be seen.

1 c 3 days after X-irradiation at 1,000 R.
The dilatation of resting cells, fragmentation of cell and round cells can be seen.

1 d X-irradiation at 1,000 R.
On 7th day after irradiating 1,000 R, cells were implanted. 4th day after implantation the giant cells can be seen.

Plate 1. Observation by means of Giemsa staining using HeLa cell after X-irradiation
after the radiation with 1,000 R, dilatation of the resting cells, fragmentation of cells and small round cells can be seen. Plate 1d shows the giant cells 4th day after implantation (on 7th day after the irradiation with 1,000 R, cells were implanted). Painter et al\(^{10}\) pointed out that the X-irradiation of HeLa cells at 1,500 R produced the giant cells and that they were independent of the metabolic cycle of DNA. Otuka\(^{11}\) reported that the index of the effect of the combined use of various antitumor substances and \(^{60}\)Co-irradiation with HeLa cells can be judged by the frequency of the appearance of the giant cells and small cells. Sheek et al\(^{12}\) are of the opinion that the formation of the giant cells by X-irradiation is due to the fact that the synthesis of DNA is incomplete. Kaplan et al\(^{13}\) stated that the cells under large irradiation become incapable of mitosis forming the giant cells.

4. Observation by Feulgen reaction

Histochemical studies were made of HeLa cells by using pyromin-methylgrün staining, PAS-staining and Feulgen reaction. Clear results could not be obtained by the two methods of staining mentioned above. However, Yabe\(^{12}\) reported that with Ehrlich cancer cells, X-irradiation increased the rate of appearance of PAS-positive cells in the cancer cells with the increase in the dose of X-rays. In our experiment, the most remarkable change was observed in the examination with Feulgen reaction. It was the picture of stickiness of chromosome. This is generally known as the primary effect of ionizing radiation, which is said to be due to the fact that the surface of the chromosome which is covered with overlapping nucleic acid is depolimerized to increase viscosity. Elkind et al\(^{15}\) reported that the sensitive sites in mammalian cells in tissue culture are the chromosomes. Recently Valencia et al\(^{16}\) have made a report that their study on the nature and frequency of chromosomal aberrations induced by X-irradiation of 2,000 R in the cells of Ehrlich ascites tumor under different oxygen tensions, revealed that the aberration observed in oxygen were of chromosome type and that those induced in anoxia were of chromatid type. This indicates the importance of the environmental conditions of the object receiving X-ray radiation. Our observation revealed that the arrangement in the chromosome was in disorder in a short time after X-irradiation, and mutually bending and stickiness took place with the passage of time. X-irradiation at more than 500 R produced in 5 to 24 hours irregularities in the arrangement of chromosome in the division stage as if each chromosome had been thrown into the cytoplasm. With the passage of time, the chromosomes in irregular arrangement fused with one another to form several DNA bulbs. They tended to combine with one another to form one to two DNA lumps. In Fig. 2 is shown the percentage of stickiness of chromosome compared with that of all other cells. It is seen from this figure that those irradiated with 200 R hardly differ from the controls and that the stickiness of chromosome is observable in 20% of those irradiated at more than 300 R, especially at 500 R and 1,000 R three to seven days after X-irradiation.

5. Observation of electron-microscopic pictures

There seems to be very few reports, on electron-microscopic study of the effect
EFFECTS OF X-RAY RADIATION ON HELA CELLS

The stickiness of chromosome X 100

<table>
<thead>
<tr>
<th>%</th>
<th>5 hrs.</th>
<th>12 hrs</th>
<th>1 day</th>
<th>3 days</th>
<th>7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>40</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Time after X-irradiation

Fig. 2. Relationship between the doses of X-irradiation and the stickiness of chromosome

of radiation on the cells of tumor. Tamagawa et al.\(^{17}\) made a report on their electron-microscopic study of the keroid caused by the atomic bomb. Urakawa\(^{18}\) stated in his report that the observation of the electron-microscopic picture of the cervix lymphatic gland of the normal white mouse subjected to the single whole body X-irradiation of 450 R revealed that this X-irradiation caused changes in the cytoplasm of the endoplasmic reticulum as well as in the nuclei and mitochondria. The results we obtained are shown in Tab. 2. The size of the non-irradiated HeLa cells is about 10 μ in section. In Plate 2 a is shown the picture of an ultrathin section of HeLa cell not irradiated with X-rays. As seen in the photograph, the cytoplasm is filled with fine cytoplasmic granules. Mitochondria (M) can be seen largely around the nucleus (N). Compared with the liver and pancreatic cells, the endoplasmic reticulum (ER) are less in number. The nucleus is surrounded by the double membrane, the cytoplasm is homogeneous and in most cases one nucleolus (Nuc) is seen inside. The observation made 12 hours after X-irradiation revealed that the cells irradiated with 200 R grew a little larger and that the cytoplasm and nucleoplasma were sporadic. With the increase in the amount of X-irradiation such as 300 R or 1,000 R, these changes tended to increase. With the X-irradiation at 1,000 R, the cells grew considerably larger and the cytoplasm was generally sporadic, especially remarkable in certain localities. In the nuclei, the nucleoplasma became sporadic and a tendency to agglomeration could be observed. Also the density around the nucleus increased. Pronounced changes were not observed in the mitochondria, endoplasmic reticulum and nucleolus. The observation made at the 3rd day of X-irradiation revealed a tendency nearly the same as that seen in the observation made 12 hours after X-irradiation, except that the changes were greater in extent than those observed 12 hours after X-irradiation. With 1,000 R irradiation in particular, the cells grew considerably larger, round degenerative portions were seen locally in the cytoplasm. Also, the agglomeration
Table 2. Electron microscopic photos of HeLa cells after X-irradiation

<table>
<thead>
<tr>
<th>The time after X-irradiation</th>
<th>12 hours</th>
<th>3 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doses of X-ray</td>
<td>Control</td>
<td>200R</td>
</tr>
<tr>
<td>Size of cells</td>
<td>about 10 ( \mu ) in section</td>
<td>a little larger</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>filled with the fine cytoplasmic granules</td>
<td>a little sporadic</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>largely around the nucleus</td>
<td>no change</td>
</tr>
<tr>
<td>Endoplasmic reticulum</td>
<td>relative few</td>
<td>no change</td>
</tr>
<tr>
<td>Nucleus</td>
<td>surrounded by the double membrane, cytoplasm homogeneous</td>
<td>cytoplasm a little sporadic</td>
</tr>
<tr>
<td>Nucleolus</td>
<td>in most cases one nucleolus in one cell</td>
<td>no change</td>
</tr>
</tbody>
</table>
of cytoplasmic granules and the increase of fat drops were observed. In the nuclei, the nucleoplasm became sporadic and tended to agglomerate, and the density around the nucleus increased. However, even at this stage, the double structure of nuclear membrane was still preserved. In Plate 2 b and c are shown the picture of the ultrathin section of HeLa cells 3 days after 1,000 R irradiation. In Plate 2 b, it is seen that the nucleoplasm become sporadic and the density around the nucleus was greater. The fat drops (F) can be seen in the cytoplasm. No pronounced changes could be seen in mitochondria and the endoplasmic reticulum. In Plate 2 c, a round degenerative site (D) is seen in the cytoplasm. The nucleoplasm is sporadic and tends to agglomerate and the density around the nucleus is great. The double structure of the nuclear membrane is still preserved.

6. Observation from respiration and glycolysis

Reports are very few on respiration and glycolysis when HeLa cells are directly irradiated with X-rays. The results we obtained from such experiments are shown
in Tab. 3. In the non-irradiated groups, respiration was 5.0 µl/10^6 cells/hr. It was 4.4 µl/10^6 cells/hr five hours after 1,000 R irradiation and 4.1 µl/10^6 cells/hr twenty-four hours after X-irradiation, showing disturbance of about 18% of non-irradiated group. In the non-irradiated group, the anaerobic glycolysis was 26.1 µl/10^6 cells/hr. It was 21.0 µl/10^6 cells/hr five hours after 1,000 R irradiation and 18.4 µl/10^6 cells/hr twenty-four hours after X-irradiation, showing disturbance of about 29% of non-irradiated group. These are almost similar to the results obtained by Prof. Ebina et al.\textsuperscript{19} from the experiment in which Yoshida’s sarcoma was irradiated with 1,000 R X-rays. According to Ord et al.\textsuperscript{20}, X-irradiation on the animal cells produced alterations to mitochondrial reactions, but such alterations develop rather more slowly than those to the nucleus, and they lead to a decrease in production of ATP and to the liberation of enzymes, such as deoxyribonuclease II. Salmon et al.\textsuperscript{21} made a report indicating that X-irradiation of HeLa cells would increase the amount of uridine diphosphoacetylglucosamine and decrease the amount of adenosine triphosphate.

7. Observation from the metabolism of nucleic acid

To the HeLa cells irradiated by the method mentioned above was added 5 µCi/ml of ^32P without any carrier (in the form of orthophosphoric acid). Through the addition of ^32P in this ratio, HeLa cells did not undergo, at least, any morphological change, and the surface dose of ^32P at the time 5 µCi/ml of it was added corresponds to about 24 rep. in 24 hours. It can be calculated from the following equation:

\[
D_p(t) = D_p(\infty) \cdot (1 - e^{-0.693 \cdot t}) \cdot (\text{MeV/g}),
\]

\[
D_p(\infty) = 79.3 \text{CE}_\gamma \cdot T \cdot (\text{MeV/g}),
\]

\[
D_p(t) = 79.3 \text{CE}_\gamma \cdot T \cdot (1 - e^{-0.693 \cdot t}) \cdot \text{rep}
\]

We determined \(D_p(t)/2\) by the above equation.

(1) Changes in nucleic acid phosphorus: Using PCA, instead of the original method by Schmidt-Thannhauser, we made fractions of RNA and DNA, and performed wet incineration of PCA and estimated it by colorimeter. The results are shown in Tab. 4 (1). A comparison of the amount of nucleic acid 3 and 15 hours after X-irradiation with that in the non-irradiation group revealed that no remarkable changes appeared either in RNA-P or in DNA-P.

(2) Incorporation of ^32P into nucleic acid phosphorus: One ml each of RNA-P and DNA-P solutions fractionized by the above method was taken to specimen
plate and, after drying it with an infrared lamp placed 40 cm away, the radioactivity was measured by GM counter (Aloka DC-5 type counting design, GM counter B 21.1 and Mica 1.6 mg/cm²). Specific radioactivity was calculated by the equation \( f_{pm} \times 10^3 \), in which \( N \) stands for N of the nucleic acid nucleic acid-Pr/mgN measured by Micro Kjeldahl method. The natural count at the time of experiment was on an average 18 cpm/min. The results are shown in Tab. 4 (2). The incorporation of \(^{32}\)P into RNA-P was inhibited more remarkably in those irradiated with X-rays than in the control.

### Table 4. 1. Change in nucleic acid phosphorus after X-irradiation

<table>
<thead>
<tr>
<th>Nucleic acid phosphorus</th>
<th>After X-irradiation</th>
<th>3 hours</th>
<th>15 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>X-irradiation of 1,000R</td>
<td>Control</td>
</tr>
<tr>
<td>RNA-P ((\gamma/mg N))</td>
<td>69.8</td>
<td>82.7</td>
<td>73.1</td>
</tr>
<tr>
<td>RNA-P ((\gamma/mg N))</td>
<td>39.8</td>
<td>31.6</td>
<td>41.8</td>
</tr>
<tr>
<td>RNA/DNA</td>
<td>1.76</td>
<td>1.97</td>
<td>1.75</td>
</tr>
</tbody>
</table>

2. Incorporation of \(^{32}\)P into nucleic acid phosphorus

\[
\text{Remark: specific radio-activity} = \frac{\text{c. p. m.}}{\text{RNA} \cdot \text{DNA} - \text{P} / \text{mgN}} \times 10^3
\]

3. Effects on mononucleotide of RNA (by use of filterpaper electrophoresis)

<table>
<thead>
<tr>
<th></th>
<th>Cytidylic acid</th>
<th>Adenyllic acid</th>
<th>Guanylic acid</th>
<th>Uridylic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (c. p. m.)</td>
<td>1250</td>
<td>350</td>
<td>368</td>
<td>223</td>
</tr>
<tr>
<td>X-irradiation of 1,000R (c. p. m.)</td>
<td>546</td>
<td>162</td>
<td>193</td>
<td>102</td>
</tr>
</tbody>
</table>

plate and, after drying it with an infrared lamp placed 40 cm away, the radioactivity was measured by GM counter (Aloka DC-5 type counting design, GM counter B 21.1 and Mica 1.6 mg/cm²). Specific radioactivity was calculated by the equation \( \frac{\text{cpm}}{\text{nucleic acid-P}_{/\text{mgN}}} \times 10^3 \), in which \( N \) stands for N of the nucleic acid measured by Micro Kjeldahl method. The natural count at the time of experiment was on an average 18 cpm/min. The results are shown in Tab. 4 (2). The incorporation of \(^{32}\)P into RNA-P was inhibited more remarkably in those irradiated with X-rays than in the control.

(3) The effect on mononucleotide of RNA: Experiments were performed by decomposing RNA into mononucleotide by the filter paper electrophoresis. That is, 0.1 ml of RNA obtained by the Schmidt-Thannhauser method was dropped on the Töyö filter paper No. 53, and fractions were obtained by use of filter paper electrophoresis. After washing in 0.1 N HCl for 24 hours, the filter used was washed in distilled water. As a buffer solution (pH 3.5), 1/50 N HCl and sodium citrate were used. After performing phoresis for five hours at 600 volt, it was
dried in a desicator, cut out into fractions by film-filter prepared by JRC and measured on the dish by a GM counter. The results are shown in Tab. 4 (3). It is seen from the incorporation of $^{32}$P into the mononucleotide of RNA that the inhibition was greater by 50% in the irradiated group than in the non-irradiated group. No remarkable difference was observed among the mononucleotides in each group. Let us now refer to the reports made by a few researchers on the effect of X-irradiation on the metabolism of nucleic acid. Mitchell(22) reports that the increase in ultraviolet absorption of the cytoplasm after X-irradiation is shown to be due to the accumulation of pentose nucleotide, probably, of ribonucleotide. In 1954 Lajtha et al(23) reported that 500 R X-ray irradiation immediately disturbed completely the composition of DNA, and several years later(24), they determined the curve of reaction caused by the difference in the amount of X-rays on the rate of composition of DNA, found that $S_1$ component abrupt in slope was present at about 500 rads irradiation and that $S_2$ component gradual in slope was present at 13,000 rads irradiation. They pointed out that $S_3$ was present due to the destruction of an enzyme and $S_2$ to the partial destruction of a chromosome. Berry et al(26) made a report indicating that although the mechanism by which X-irradiation inhibits synthesis of DNA is still unexplained, the ratio sensitivity had much to do with the presence of oxygen. Fernendegen et al(28), using HeLa cells, confirmed the following: Cytidine is in part converted to thymidine for DNA synthesis and uridine for RNA synthesis. Also RNA synthesis begins in the chromatin portion of the nucleus. Recently Painter(27) has reported that in HeLa $S_3$ cell cultures, after X-irradiation, there is an immediate depression of the rate of DNA synthesis, but there is no similar depression in RNA synthesis.

REFERENCES


