Depigmentation of Hair in C57BL Mice after Injection of Irradiated Thymic RNA

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Ribonucleic acid extracted from the thymus of C57BL mice by the sodium dodecyl sulfate-phenol method was irradiated with 10^6R of Co-60 γ-rays. After the exposure, Sephadex column chromatography revealed that 27.7 per cent of original RNA had been decomposed to smaller molecules with molecular weights below 10,000. The irradiated solution was then injected subcutaneously to new-born mice of the same strain. White hairs were recognized in the fur of the mice injected with irradiated RNA solution, but not in the animals which received non-irradiated RNA. The difference in the proportion of depigmented hairs between the two groups was statistically significant. In later hair generations, white hairs were replaced by darker ones. A variation in the pigment content of cortical cells of dark hairs was also observed.

Depigmentation of hair after irradiation has long been accepted as an established fact since Hance and Murphy's report in 1926. Its relation to radiation dose was investigated quantitatively by Chase. The mechanism of depigmentation is, however, still obscure.

The effects of ionizing radiation on nucleic acids such as breakage of internucleotide linkages and hydrogen bonds, or destruction of bases and cross-linking of polynucleotide chains have been intensively studied by numerous workers. These changes in nucleic acid are considered to play an important role in radiation effects on biological systems. However, it still remains to be examined how the changes in nucleic acid can bring about biological effects. Attempts at elucidating this mechanism as in the reports of Lauffer et al. and Englander et al. dealing with inactivation of tobacco mosaic virus by breakdown of nucleic acid have hitherto been described only in a small number of publications.

In the present experiment, an attempt was made to determine whether the change in RNA molecules by irradiation is one of the underlying processes in the depigmentation of hairs. Ribonucleic acid (RNA) extracted from the mouse thymus was exposed to γ-ray in vitro and then injected subcutaneously to new-born mice of the same strain, and its effect on the color of growing hair was investigated.

Received for publication, March 30, 1967.
MATERIALS AND METHODS

Preparation of RNA solution. For each experiment, RNA was extracted by sodium dodecyl sulfate-phenol method\textsuperscript{13,14} from 20 thymuses of C57BL mice of both sexes of 30 to 40 days of age. At this age the weight of the thymus is largest throughout the whole life. After minced well with scissors, the organs were homogenized with a Potter teflon homogenizer for 3 min with cold Takanami’s buffer (0.25 M sucrose, 0.005 M \text{MgCl}_2\cdot6\text{H}_2\text{O}, and 0.025 M KCl in 0.05 M Tris buffer at pH 7.6). Nuclei were removed as precipitate by centrifugation at 800 g for 10 min. The sediment was washed twice by resuspension in the homogenizing solution. Combined supernatant fluid was stirred after sodium dodecyl sulfate (SDS) was added to make a final concentration of 0.5%. At this concentration this substance is known to inhibit ribonuclease perfectly. Before the addition of SDS the procedure was carried out at 0°C. An equal volume of water-saturated phenol was then added and the mixture was shaken for 3 min at room temperature. The emulsion was then broken by centrifugation at 20,000 g for 1 min and the extraction was further repeated twice on the aqueous phase. RNA was precipitated from the aqueous phase by addition of 2 volumes of cold ethanol and redissolved in pH 5.1 acetate buffer containing 0.05 M NaCl and 10\textsuperscript{-4} M Mg\textsuperscript{2+}. Polyvinyl sulfate (PVS), an inhibitor of ribonuclease, was added at this step. RNA samples were then incubated with DNase II (15 μg/ml) for 60 min at 37°C to remove contaminant DNA.

Irradiation. The RNA solution in a test tube was irradiated with 10\textsuperscript{6} R in a Co-60 apparatus of 5,000 Ci at the Department of Nuclear Engineering, Tohoku University. The dose rate was 2.77\times10\textsuperscript{5} R/hour at 11.5 cm of source-to-sample distance. Irradiation was carried out at 0 to 5°C and the RNA solution was air-bubbled during the exposure; the RNA concentration was about 150 μg/ml.

Column chromatography of RNA. Soon after the irradiation, 1 ml of the RNA solution was layered onto a 12 cm Sephadex G-50 column and fractionated with pH 5.1 acetate buffer. Sephadex G-50, the dextran mesh, retains only molecules smaller than 10,000 in mwt and larger molecules are eluted first from the column. Each fraction consisted of 1.1 ml and its ultraviolet absorption at 260 nm was measured by a Hitachi spectrophotometer after addition of 2 ml of acetate buffer.

Injection. One-tenth ml of RNA solutions which contained about 15 μg RNA irradiated or non-irradiated was injected subcutaneously into the back of a newborn mouse within 20 hours after the birth. C57BL/6 mice had originally been supplied by National Institute of Animal Health and had been reproduced in our department. The mouse of this strain has black hair coat, and white hairs appear sparsely among black ones only after the age of 7 months. New-born siblings were divided into two groups. In one group, whole irradiated RNA solution was injected to the animals. In the other group, which was used as the control, only large molecular fraction of non-irradiated RNA was given after equalization of RNA.
Depigmentation of Hair by Irradiated RNA

Animals of both groups were weaned 25 days after birth and fed thereafter on CMF pellet (Oriental Yeast Co.) and water ad libitum. Five or six animals were reared in each cage.

Histological examination. Hair was fixed in 10% formalin, embedded in paraffin, sectioned in 7 μ thickness, and stained with hematoxylin-eosin, Masson's trichrome and Fontana's silver reagent following Masson's ammoniacal silver technique.

RESULTS

1) Degradation of RNA molecule by irradiation

The procedure of RNA extraction, irradiation and column chromatography was repeated 9 times; a half of the solution was irradiated and the rest was kept as control. Elution patterns of irradiated or control RNA from a Sephadex column is illustrated in Fig. 1: the first sharp peak on the left represents large molecular RNA above 10,000 in mwt, which diminishes in quantity after irradiation. The comparison with non-irradiated RNA yielded the results shown in Table 1. These are expressed as per cent of the control in each experiment. The total RNA amount at the peak of large molecules (Σ optical density × 3.1 ml) of irradiated solution was 72.3% of that of control. This indicates that about 30 per cent of original RNA have degraded into smaller molecules below 10,000 in mwt by irradiation of 10⁶R. RNA concentration was calculated by dividing optical density at 260 mμ by a factor 25.3 ml/mg. Destruction of bases or cross-linking cannot be demonstrated with this method of Sephadex column chromato-

![Fig. 1. The elution pattern of RNA from G-50 Sephadex column with acetate buffer pH 5.1.](image)

The first sharp peak of large molecule is lowered by irradiation.
TABLE 1. The degradation of RNA molecules by γ-irradiation of 10^6 R

<table>
<thead>
<tr>
<th>No. of experiment</th>
<th>The amount of large molecule RNA</th>
<th>Per cent of control</th>
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<tbody>
<tr>
<td></td>
<td>Irradiated</td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>61.3</td>
<td>96.1</td>
</tr>
<tr>
<td>2</td>
<td>87.0</td>
<td>120.9</td>
</tr>
<tr>
<td>3</td>
<td>83.2</td>
<td>116.1</td>
</tr>
<tr>
<td>4</td>
<td>61.0</td>
<td>89.3</td>
</tr>
<tr>
<td>5</td>
<td>60.8</td>
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<td>101.0</td>
</tr>
<tr>
<td>8</td>
<td>73.1</td>
<td>106.4</td>
</tr>
<tr>
<td>9</td>
<td>67.5</td>
<td>74.2</td>
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72.3±9.6%

graphy, but the method provided reproducible estimations as for the change in the dimension of RNA molecules.

2) Depigmentation of hair

White hairs were observed in the first growing hair coat of the mouse injected with irradiated RNA solution. An example at the age of 4 weeks is shown in Fig. 2.

Fig. 2. The upper mouse which received irradiated RNA at new born period has white hairs in 30%. But the lower animal injected with non-irradiated RNA has not.

Fig. 2 in comparison with a control mouse. Depigmented hairs were more prominent on the back than in other parts of the body, appearing not in bundles but dispersedly among black hairs. More than 500 hairs were cut from
Depigmentation of Hair by Irradiated RNA

TABLE 2. Number of mice which have white hair or not at various intervals after the injection. The difference in the appearance of white hair was tested between the experimental and control groups.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Control 1-6 months after non-γ-rayed RNA</th>
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<tr>
<td>Month after the injection of γ-rayed RNA</td>
<td>1</td>
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<tr>
<td>White hair(+)</td>
<td>13</td>
</tr>
<tr>
<td>White hair(−)</td>
<td>18</td>
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<tr>
<td>Total</td>
<td>31</td>
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<table>
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<tr>
<th>$\chi^2$</th>
<th>10.77</th>
<th>5.0</th>
<th>1.96</th>
<th>0.65</th>
<th>0.58</th>
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<tr>
<td>DF</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Difference (α=0.05)</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
</tbody>
</table>

Fig. 3. Back hairs of a 6 month old mouse which received irradiated RNA. Various degree of pigment content is observed in the cortical cells of hairs. No staining. × 100.

the back of an animal and mounted on slide glass with glycerin. When animals had white hairs in more than 10% in their hair coat, the results were regarded as positive. The proportion of the mice with white hairs at various intervals after the injection are demonstrated in Table 2. Sixty-four new-born mice of both sexes were administered RNA solution, but only 50 survived more than 4 weeks because of a failure in the nursing. In the animals of experimental group, which were given irradiated solution, 13 out of 31 mice had white hairs at the age of 1 month. On the other hand, depigmentation of hairs was observed in none of 19 mice of the control group injected with non-irradiated RNA solution. In later hair generations, however, the percentage of white hair decreased, showing that white hairs were apparently replaced by darker ones. The decreasing rate of animals with depigmented hairs could be approximated by an exponential function of months after RNA injection. The difference between the experimental and control groups
Fig. 4. Club hairs in resting follicles of telogen stage. The hair of Plate B has black melanin pigment in the cortex cells above the surface of the skin, but the white hair in Plate A has pink material stained by eosin instead of black pigment. Hematoxylin-eosin staining. × 250.

was statistically significant in 1 and 2 months, but not significant in 3, 4 and 6 months after the injection. One mouse died of pneumonia at 3 months of age, and undergrowth of animals had otherwise been observed. There existed various degrees of pigment content in the cortical cells of hairs as indicated in Fig. 3. Fig. 4 shows the histological section of a club hair in a resting follicle of telogen stage. No difference was observed between follicles of white and black hairs except for their
content of melanin pigment. Cortical cells of white hairs had pink granules stained by eosin instead of melanin pigment.

**DISCUSSION**

Degradation of RNA molecules by irradiation was detected with Sephadex column chromatography. Injection of this degraded RNA caused white hairs to appear in originally black haired mice C57BL. No such white hairs were observed in the mice of the control group that were administered non-irradiated RNA. The difference in the proportion of grey hair coat was statistically significant between the two groups. The induction of white hair will be attributed to irradiated RNA, because the treatment was quite the same in the two groups except that the injected RNA was exposed or not.

As to the effect of irradiation on depigmentation of hairs, two major hypotheses have been advanced. In one of them direct injury or inactivation of melanoblasts is regarded as one of the important factors. The other presumes indirect inactivation of melanocytes. Cohen demonstrated clearly by the tissue culture method the presence of a competent pigment cell system in explants from whitened feather collar brought about by irradiation. He assumed that the pigment cell system of such explants is already potentially patent to produce melanin, although the pigment formation was not actually demonstrated. Chase's paper also presented the evidence for the indirect effects by means of short-range heavy ions. Both authors are of the opinion that radiation depigmentation is the result of indirect inactivation of melanocytes mediated by ectodermal cells. The extent of pigment production seems to depend upon the control of associated ectodermal cells. Our study also presented the evidence for indirect effect of γ-exposure: melanocytes were not irradiated, but only RNA was irradiated in vitro. It seems likely that RNA degraded by irradiation impairs the normal control of pigment formation in melanocytes.

**References**


3) Kamata, R. Radiation effect on the hair tissues. *Ochanomizu Igaku Zassi* (Jap.), 1958, 6, 218-238.


**Acknowledgment**

We are indebted to Dr. A. Banno, Department of Nuclear Engineering for the irradiation, to Dr. T. Ino of National Institute of Animal Health for the supply of (57 BL mice), and to Prof. T. Mori, Department of Anatomy, and Dr. Y. Sasai, Department of Dermatology, Tohoku University for the histological examinations.