THE INFLUENCE OF ROENTGEN RADIATION ON IMMUNITY TO SHOPE FIBROMA VIRUS

JOHANNES CLEMMESEN
(From the Department of Experimental Pathology and Cancer Research, University of Leeds)

Various authors have demonstrated that a general exposure of mice and rats to roentgen radiation will decrease their resistance to subsequent inoculation of a tumor or a leukemia. A review of these communications has been given in a recent publication (1938). Moreover, it has been shown (1937, 1938) that the effect of the radiation is probably due to a delay in the development of immunity to foreign cells, at least so far as heterologous transfer of tumors is concerned. A decreased resistance to bacterial infection was demonstrated in x-rayed mice by Chrom (1934, 1935), who ascribed this effect to an influence on the reticulo-endothelial system.

The object of the present study was to ascertain whether a similar decrease in resistance could be demonstrated when a virus (Shope fibroma) was inoculated into irradiated rabbits. This was established, and it was shown that the effect must be explained as a delay of the development of immunity to inoculations of the virus employed, probably due to a temporary inactivation of the reticulo-endothelial system.

Author's Experiments

The reaction of normal domestic rabbits to inoculation of Shope fibroma virus is to some extent dependent on the site of the inoculation. Thus intradermal injections will regularly result in the development of tumors, which after about two weeks regress and finally disappear entirely, leaving an immunity against subsequent inoculations. Tumors developing as a result of successful intramuscular injections, however, are as a rule larger and of more variable size than the intradermal fibromata. Intravenous injections of Shope fibroma virus into normal rabbits have so far been without positive results.

Technic: In the following experiments the injections of virus were made in rabbits which had undergone roentgen irradiation during the preceding twenty-four hours.

The irradiations were done by means of a Multi-Stabilivolt apparatus from the General Radiological and Surgical Apparatus Ltd., under the following conditions: 200 kilovolts, 10 milliamperes, 1 mm. copper + 0.5 mm. aluminum filtration, half-value layer 1.48 mm. copper. As a rule, the rabbits were exposed two at a time, in a wooden box with a horizontal partition. The dimensions of the box were 40 × 30 × 30 cm. and the thickness of the walls 1 cm.

Irradiation was given to the side of the animals with a distance of 80 cm. from the focus to the wall of the box. When half the dose had been given, the

1 An extension of a communication given at the VII Scandinavian Pathological Congress, June 30, 1938, Copenhagen.
IMMUNITY TO SHOPE FIBROMA VIRUS

box was turned so as to expose the other side of the animals. In this way homogeneous irradiation throughout the body was obtained. Measurements, with a Siemens intensimeter, at either end of a dummy filled with rice flour, gave an intensity 16 r and 6 r per minute respectively. Thus the intensity in the center of the rabbit was estimated to be 11 r per minute. The full dose varied between 300 and 700 r.

The virus employed was one of the OA strain and was kindly provided by Dr. C. H. Andrewes and Dr. L. Foulds. Material for inoculation was obtained from testes of inoculated rabbits. As a rule, the testes were dried in a desiccator and were prepared for inoculation by grinding with sand in a mortar. In some cases, however, the solution was prepared from an extract of testes kept in 50 per cent glycerine. The concentration used for intradermal and intramuscular injections was about 5 per cent, and the doses were respectively 0.2 and 2.0 c.c. For intravenous injections the testes were stored for twenty-four hours only, in 50 per cent glycerine, and the dose was 5 c.c. of a 10 per cent solution. So far as possible, controls and experimental animals of the same sex and color were chosen, though these factors were apparently without influence on the results.

Intradermal Injections: Figs. 1–3 show the development of the Shope fibroma in x-rayed rabbits and normal controls. Each rabbit received three injections. The average value of the two greatest diameters of the largest

---

**Fig. 1. Production of Shope Rabbit Fibroma in Irradiated Animals and Normal Controls Following Intradermal Injection of the Virus**

In this and Figs. 2 and 3 the heavy line represents the irradiated animals, the lighter line the controls.
nodule is given as ordinate. The abscissa shows the time in days (1 cm. = 2 days). The circular spot represents the dimensions of the skin tumor at the end of the experiment, after the number of days given near it.

It is seen that there is a marked difference between the tumors in x-rayed and normal rabbits. The tumor attains a greater maximum size in the ir-

![Diagram](image-url)

**Fig. 2. Production of Shope Rabbit Fibroma in Irradiated Animals and Normal Controls Following Intradermal Injection of the Virus**

radiated animal, and usually reaches this at a later point of time than in the control, due apparently to the fact that the growth continues longer.

The results recorded were obtained in 11 x-rayed rabbits and 11 controls. Seven of these pairs were also injected intramuscularly and one intratesticularly. The effect obtained by irradiation was perhaps less pronounced in these cases, but was still unmistakable except in two cases where the difference was only slightly in favor of the irradiated animal.

*Intramuscular Injections:* Table I shows the results of intramuscular injections of Shope fibroma virus in x-rayed rabbits. All these animals received intradermal injections at the same time.
Although measurements could not be carried out with the same accuracy as with intradermal fibromata, it is obvious that the conditions of growth were much better in the irradiated rabbits than in the controls.

*Intravenous Injections:* According to previous investigations (Andrewes, Ahlström, Foulds and Gye, 1937; Ahlström and Andrewes, 1938) intravenous inoculation of Shope fibroma virus does not produce any visible change in ordinary normal rabbits apart from a small nodule at the site of injection and occasionally a few transient skin "pocks" (Andrewes, 1936).

An experiment was undertaken to investigate the result of intravenous inoculation into x-rayed rabbits. It must, however, be pointed out, that while previous authors have used a 5 per cent solution, a concentration of about 10 per cent was employed in this study. The results are recorded in Table II.

Fig. 4 shows the liver from Rabbit 91, in which this organ was the site of the most pronounced changes. In the other cases the size of the liver nodules was only a little larger than a pin-head. With the exception of No. 91 the nodules in this experiment seemed on the whole smaller than those described by Ahlström and Andrewes (1938), although localization and histological structure were typical.

*Immunity to Shope Fibroma Virus in Irradiated Rabbits:* With the intention of determining whether the effect of roentgen radiation might be explained as a delay of the development of immunity to the virus, the following experiment was planned. Six x-rayed rabbits and 6 controls received daily intra-
TABLE I: Maximum Size of Fibromata in Irradiated and Normal Rabbits Following Intramuscular Injections of Virus

<table>
<thead>
<tr>
<th>Rabbit No.*</th>
<th>Day of Measurement</th>
<th>Tumor Dimensions (in cm.) in Irradiated Rabbits</th>
<th>Tumor Dimensions (in cm.) in Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>28th to 33rd</td>
<td>12.5X10X3.5</td>
<td>3.5X3.5X3.5</td>
</tr>
<tr>
<td>17</td>
<td>28th to 33rd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>30th</td>
<td>4X4X1.5</td>
<td>Small nodule</td>
</tr>
<tr>
<td>19</td>
<td>30th</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>19th</td>
<td>1.5X1.5X0.75</td>
<td>Small nodule</td>
</tr>
<tr>
<td>21</td>
<td>19th</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>32d</td>
<td>8X6X3.5</td>
<td>1.5X1.5X1.5</td>
</tr>
<tr>
<td>23</td>
<td>21st</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>24th to 32d</td>
<td>8.5X7.5X3.5</td>
<td>4.5X3.5X2.5</td>
</tr>
<tr>
<td>25</td>
<td>15th to 30th</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>32d</td>
<td>6.5X4X2</td>
<td>No nodule</td>
</tr>
<tr>
<td>27</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Rabbits 16 and 18 received 300 r; rabbits 20, 22, 24, and 26 received 400 r.

TABLE II: Results of Intravenous Inoculations of Shope Fibroma Virus in Heavily X-rayed Rabbits and Normal Controls

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>X-rayed Animals</th>
<th>Normal Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>58</td>
<td>700 r Fibromata of back, paws, penis, and testis</td>
<td>No visible change</td>
</tr>
<tr>
<td>57</td>
<td>600 r Fibromata of back and paws</td>
<td>No visible change</td>
</tr>
<tr>
<td>71</td>
<td>600 r Fibromata of back, paw, testis, jaw, and liver</td>
<td>No visible change</td>
</tr>
<tr>
<td>72</td>
<td>600 r Fibromata of back, paw, and liver</td>
<td>Small subcutaneous nodule</td>
</tr>
<tr>
<td>87</td>
<td>600 r Fibromata of back, paw, and liver</td>
<td>No visible change</td>
</tr>
<tr>
<td>88</td>
<td>600 r Fibromata of back, paw, and liver</td>
<td>No visible change</td>
</tr>
<tr>
<td>89</td>
<td>600 r Fibromata of back, paw, and liver</td>
<td>No visible change</td>
</tr>
<tr>
<td>90</td>
<td>600 r Fibromata of back, paw, and liver</td>
<td>No visible change</td>
</tr>
<tr>
<td>91</td>
<td>600 r Fibromata of back, paw, and liver</td>
<td>No visible change</td>
</tr>
</tbody>
</table>

dermal inoculations of Shope fibroma virus. After a latent period fibromata developed in a number corresponding to the number of days elapsed before the development of full immunity. Fig. 5 shows the result. It is seen that immunity developed at a much later point of time in x-rayed than in normal rabbits.

Other experiments on 6 rabbits proved that immunity, if once developed, cannot be broken down by means of roentgen irradiation.

DISCUSSION

There is a considerable similarity between the observations here reported and the results which Andrewes, Ahlström, Foulds and Gye (1937) and Ahlström and Andrewes (1938) obtained with tarred rabbits inoculated with
Shope fibroma virus. These authors found that Shope fibromata continued their growth for a longer time in rabbits treated with injections of tar or certain carcinogenic hydrocarbons. One phenomenon observed by them, however, namely the occurrence of sarcomata in tarred rabbits, has no parallel in the present material, but this may be due to the fact that most of the irradiated rabbits in this series died a relatively short time after the beginning of the experiment.

To this comparison between x-rayed and tarred rabbits the objection might perhaps be raised that Ahlström and Andrewes (1938) failed to demonstrate any delay in the development of immunity in tarred rabbits similar to the inhibition recorded here in x-rayed animals. This difference is only apparent, however, as even in my material a comparison between experimental and control animals would fail to show any difference if undertaken at the point of time when Ahlström and Andrewes made their observation.

This similarity between tarred and x-rayed animals is not without parallel. It has been mentioned that mouse tumors inoculated into x-rayed rats will continue their growth for a longer period than in normal rats, just as homologous transplanted tumors are favored in x-rayed animals (1938). Correspondingly it was found by Carrel (1925) and by Maisin and Masse (1925) that when minced chicken embryo was injected into hens treated with injections of tar, the embryomata which developed grew to a larger size and for a longer period than in untarred birds.

Mayneord and Parsons (1937) injected carcinogenic compounds into x-rayed mice and found a higher incidence and more rapid development of tu-
mors than in controls. Maisin and Masse (1925) made similar observations following tar painting of mice previously given injections of tar.

It may now be justifiable to set up a third parallel. Just as transplantation of cells, whether homologous or heterologous, is favored by roentgen radiation and tar injections, so it is also favored by treatment with trypan blue (Ludford, 1931). Finally Ludford has found (unpublished experiments) that intradermal Shope fibromata in rabbits treated with trypan blue (0.5 per cent, 6–9 c.c. × 7) will grow and persist for a longer period than in normal rabbits. It seems in this connection to be of some significance that trypan blue as well as roentgen radiation is generally considered a reliable means for influencing the reticulo-endothelial system. Thus it may be a justifiable assumption that injections of tar, as well as roentgen irradiation and treatment with trypan blue, exert their influence on tumor growth through the reticulo-endothelial system. It may even be possible by means of roentgen irradiation to favor heterologous transmission of certain viruses.

**Summary**

Shope fibroma virus injected into rabbits treated with a general roentgen irradiation produces results similar to those described by Andrewes and others in rabbits injected with tar, namely prolonged growth of the resultant fibromata, prolonged resorption, and generalized fibromatosis after intravenous inoculation.

The development of immunity to repeated inoculations with this virus is delayed in x-rayed rabbits.

The effects of a general roentgen irradiation on transplantation of tumor and normal cells, inoculation of fibroma virus, and artificial carcinogenesis, have their parallels in the effects of injections of tar and of trypan blue.
It is suggested that all these procedures act through the reticulo-endothelial system.

I have much pleasure in thanking Drs. la Touche and Spiers for their kind assistance at the arrangement of the irradiations, which were carried out at Leeds General Infirmary.

**Literature**


Chrom, S. A.: Roentgenbestraalings Indvirkning paa Infektionsmekanismen hos hvide Mus, Copenhagen, 1934.


