THE EFFECT OF X-RAY ON THE SKIN OF VITALLY STAINED WHITE MICE.

ESTABLISHMENT OF AN X-RAY UNIT FOR THE SKIN OF THE MOUSE.

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Numerous experiments have been carried out for the purpose of sensitizing the body toward the visible and ultravisible rays of the sun and artificial light sources (Hausmann (1)). Yet little is known about the sensitization of the animal body towards x-ray.

Ellinger (2) injected rats subcutaneously with a 10 per cent solution of thorium nitrate and found that a smaller amount than usual of subsequent radiation led to marked ulceration of the skin. Similar experiments on cancer patients, in which the tumor itself was infiltrated with the thorium salt, led to a disintegration of the tumor after a radiation with one tenth to one half of the erythema dose. Baldwin (3) found that about one half of the x-ray energy that killed normal paramecia was sufficient to kill vitally stained paramecia. He repeated these experiments on metazoa, injecting white rats with trypan blue. Baldwin found that while the unstained rats died within 100 to 120 hours after radiation, the stained animals survived only 60 to 70 hours. He explains the sensitization of the tissues towards x-ray by an absorption of the stain on the surface of the cell nucleus, a phenomenon which occurs, as he says, most actively under the direct influence of an electromagnetic form of energy.

The following experiments were undertaken to investigate whether the skin of vitally stained white mice is more sensitive toward x-ray than the skin of normal mice. In connection with these experiments an attempt was made to establish a unit of x-ray energy for the skin of the mouse, which could be compared with the human erythema dose. Since an x-ray erythema does not develop on the skin of the mouse, the spontaneous total epilation of the radiated area was chosen as a unit.

EXPERIMENTAL.

White, male, full grown mice of a single strain were used exclusively. The animals were kept in pairs in small cages and fed on dog biscuits and water. One of each pair received the injection of the stain, the other served as a control. Trypan blue (Grübler) was chosen for the vital staining. The stain was made up to a concentration of 0.5 per cent with normal saline. The mice received a subcutaneous injection of 0.5 cc. of this solution on the morning of the 1st, 3rd, and 5th day. On the afternoon of the 5th day the mice were rayed. The legs were fastened with clamps so that the animal could not move and the body was covered with lead sheets, leaving free a field of 1.0 × 1.5 cm. on the left hind part of the back. The control mice were rayed in exactly the same way. A Kelly-Koett apparatus with a Coolidge tube was used. A radiation of 4 milliamperes, 95 kilovolts at a skin target distance of 22 cm. with an aluminum filter of 2.5 mm., was given. The human erythema dose using this combination for a field of the same size is 12 minutes. Fifty animals, of which twenty-five were stained and twenty-five served as controls, were radiated. Groups of ten received a radiation of 80, 70, 65, 60, and 50 minutes.

It was found that the reaction of the radiated skin in stained and control mice was not markedly different, the sensitiveness of that of the stained mouse being apparently slightly below that of the control mouse. There was, however, a marked difference in the time at which epilation began after radiation. Data on this point are summarized in Table I.

It can be seen that the epilation occurred earlier in the stained than in the control mice, rayed for the same length of time. The 80 and 70 minute radiation caused in all a skin ulceration which occurred a few days after the beginning of the epilation. The ulcer was subsequently covered with a crust and finally a scar was formed. A new growth of hair in the radiated region never followed. The skin on the ventral part of the trunk opposite to the rayed area showed a complete epilation without ulceration. This area received about 70 per cent of the rays, since the tissues they had to pass were about 1 cm. thick. The 65 minute radiation caused total epilation in the dorsal and incomplete in the ventral field. All five control mice of this group developed a small ulceration on the dorsal skin, while out of the five stained ones, only three had superficial ulcerations. A new growth of hair took place in several instances on the ventral field. The 60 minute radiation caused complete epilation and a

slight superficial ulceration in three out of the five controls and in one out of the five stained mice. The ventral field was unaffected.

The 50 minute radiation caused no epilation either in vitally stained or control mice. Nearly all the mice were kept under observation for 3 months after radiation.

The epilation dose for the normal mouse lies between 50 and 60 minutes with the radiation combination above described. This

TABLE I.

Time interval between radiation and the occurrence of epilation.

80 min. radiation.		70 min. radiation.	
Control mice.	Stained mice.	Control mice.	Stained mice.
days	*days*	*days*	*days*
16	15	18	16
16	10	20	14
18	10	18	11
17	11	20	13
22	14	21	14
Average 17.8	12	19.4	13.6
65 min. radiation.		**60 min. radiation.**	
18	15	16	16
21	14	23	14
20	12	22	22
17	12	26	21
17	13	24	20
Average 18.6	13.2	22.2	18.6

amount should cause total spontaneous epilation without any ulceration, but the individual variability has to be taken into consideration. Ten mice were radiated for 60 minutes. For four mice this proved the exact epilation dose, while for four it was a dose slightly above the epilation dose, since superficial ulceration was observed. The two other animals developed only incomplete epilation.

The epilation dose for mice is about four to five times more than the human erythema dose, and corresponds closely to that which Wood

and Prime (4) had to apply in order to kill mouse carcinoma outside the body; Seitz and Wintz (5) have shown that in human beings the ratio of erythema dose to carcinoma dose is about 100:120. It has since been found that this does not hold true for every type of human tumor. The argument that huge doses of x-ray are required to destroy mouse carcinoma is obviously fallacious, because the human erythema dose has no significance for the mouse. Since apparently there is a difference in the sensitiveness toward x-rays of the skin in different species, the epilation dose of each species has to be taken as basis and not the human erythema dose. Experiments are in progress to establish the relationship between the epilation dose and the dose which destroys mouse carcinoma *in vivo*.

Kok and Vorlaender (6) determined a skin dose for the mouse. They termed epilation dose the amount of x-rays which made it possible to pull out easily the hair in the radiated region after a certain length of time. This dose amounts to one to two human erythema doses. But the force necessary to pull out hair easily is arbitrary and cannot well be standardized. The authors mentioned state further that much larger doses caused no ulceration of the skin of the mouse. Consequently the epilation dose established in the present work which is based on spontaneous epilation, is much greater than that of Kok and Vorlaender.

SUMMARY.

1. The time interval between radiation and the occurrence of epilation is shorter in mice injected with trypan blue than in normal animals.

2. An x-ray unit defined as that causing total spontaneous epilation on the skin of the mouse is suggested. It corresponds to four to five human erythema doses.

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