

## THE EFFECT OF X-RAY IRRADIATION ON MELANOCYTES IN THE SKIN\*

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The effect of x-rays on skin is described in most textbooks of dermatology. However, the literature contains few reports of their action on melanin pigmentation of the skin. X-rays are known to produce degenerative changes in all tissues. Their effect is apparently proportionate to the mitotic activity of the cellular structure. After a brief latent period, shorter with heavy dosage, living cells manifest progressive turbidity and granular alteration of their cytoplasm, lose their motility, and cease mitotic activity. After a period of inactivity, they may resume a normal function, or they may suddenly die; or their granular dissolution may occur promptly if dosage is heavy. Sutton (1) describes how in human skin, in a mild acute reaction, there is redness which is accompanied by a tenderness, burning and itching, which disappear in two or three weeks, leaving temporary pigmentation. In chronic cases, the skin becomes thin, dry, atrophic, wrinkled, telangiectatic and pigmented. The object of the present work was to study the effect of x-rays on the melanocyte numbers and their morphology using a carefully controlled histochemical experiment.

### MATERIALS AND METHODS

Twelve mature male guinea-pigs, weighing between 365 and 725 gms. were used. They were divided into two groups, each consisting of three pure black and three pure red animals. The anterior abdominal wall of each animal was shaved and a control biopsy specimen of skin was removed from just below the costal margin. When the biopsy wound had healed, the skin of the lower part of the anterior abdominal wall was exposed to x-ray irradiation. The procedure was as follows: Each animal was securely held with the anterior abdominal wall facing upwards, as shown in fig. 1. The x-rays were delivered at 50 K.V., 2 cms. F.S.D., with a surface dosage of 500 rads. (giving a dose of 400 rads. at one mm. depth) in 0.07 of a minute. The irradiation was administered to the skin of the right iliac fossa at three sessions at weekly intervals. The first group of animals were killed twelve days after the last treatment, and those of the second group were killed nineteen

days after the treatment; a second skin specimen was then removed from the affected area.

All the skin specimens were processed under exactly identical conditions, as described elsewhere (Snell (2)), and were subjected to the dopa reaction. The greater part of each skin sheet was mounted in Depex; the remainder was embedded in paraffin and vertical sections  $6\mu$  thick were cut. The skin sheets were examined with the epidermal surface uppermost. The effect of x-ray irradiation on the melanocytes was studied by comparing the specimens removed before and after treatment. The appearances of the melanocytes were studied by assessing the number and size of the melanocytes and the amount, color and position of the melanin within the cells. The length, width and complexity of the dendritic processes were also noted, and an attempt was made to assess the amount and color of the free melanin present, *i.e.* that melanin which is situated outside the melanocytes. The melanocytes were counted in ten areas chosen at random, each measuring 0.07 sq. mm. The counts were carried out using a graticule fitted in the eye-piece of a microscope and a magnification of  $\times 353$  was used.

Kodacolor photographs were taken of the anterior abdominal wall skin of the red animals before and after the experiment. The conditions were standardized using a fixed magnification and light intensity. Color balance was checked by the use of a grey scale and color patches introduced at each photographic session.

### RESULTS

The melanocytes of guinea-pig skin are found in two sites, the hair follicles and the basal layer of the epidermis. In the present work, the study has been confined to the melanocytes and melanin of the surface epidermis. The appearances of the melanocytes and melanin in the skin of the normal guinea-pig at different ages in the two sexes have been reported previously (Bischitz and Snell (3), Snell and Bischitz (4)). The proof that in the normal untreated guinea-pig, the melanocytes and melanin in the two biopsy specimens of anterior abdominal wall skin, which were removed before and after the experiment, are for practical purposes identical has already been described (Snell and Bischitz (5)).

### MACROSCOPICAL OBSERVATIONS

No erythema, edema or gross exfoliation of the skin was noted in any of the guinea-pigs. On the

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seventh day following the first administration of x-rays, a slight increase in the degree of pigmentation in the anterior abdominal wall skin had occurred in the red animals and this became

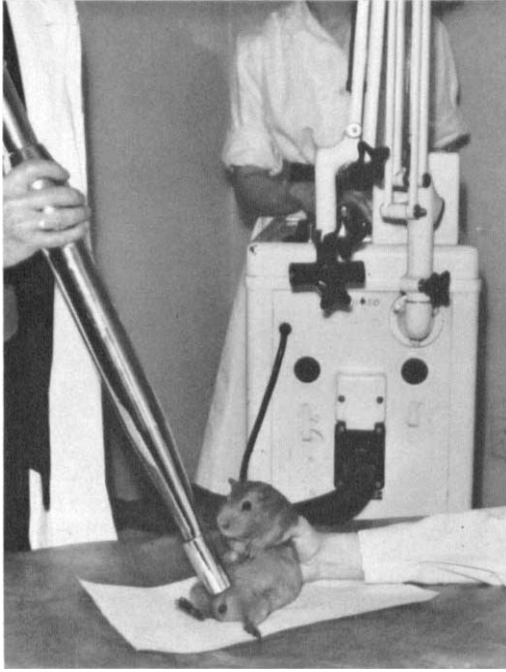


FIG. 1. Photograph of a guinea-pig receiving x-ray therapy.

progressively darker as the treatment continued (fig. 2). At the end of the experiment the increase in pigmentation was so great that it was possible for it to be detected in the black animals. In guinea-pig No. 1147, however, the increased pigmentation did not appear until twenty-one days had elapsed after the first treatment. At the end of the experiment, it was noted that the hair growth was absent in the treated area in all the animals.

#### MICROSCOPICAL OBSERVATIONS

The detailed results are recorded in Table I. In Group A (killed twelve days after last treatment), the amount of melanin present both inside and outside the melanocytes showed a considerable increase (fig. 3). This was accompanied by an enlargement in the cell body size and an increase in the length, width and complexity of the dendritic processes. In the red animals, the depth of color of the melanin was also increased. In Group B (killed nineteen days after last treatment), the effect was seen to be similar (figs. 4 and 5) but the increased amount of melanin present within the melanocytes was seen to be less than that which occurred in Group A, and the amount of free melanin was also slightly smaller. A comparison of the results of Group A and B in the table shows a correspondingly diminished increase in the cell body size and in the



FIG. 2. Kodacolor photographs of the anterior abdominal wall of a red mature male guinea-pig (No. 1152); (a) before x-ray irradiation, (b) after x-ray irradiation. Shows a very great increase in the pigmentation of the anterior abdominal wall. The size and shape of the pigmented area were governed by the size and shape of the aperture of the applicator.

TABLE I  
Showing the effect of x-ray irradiation on the melanocytes and melanin in the skin of the anterior abdominal wall of the male guinea-pig

Number of Animal	Color of Animal	Appearances of Melanocytes										Appearances of Free Melanin							
		Cell body					Dendritic processes					Amount	Depth of color						
		Size	Amount of melanin	Position of melanin	Depth of color	Length	Complexity	Width	Amount of melanin	Arrangement of melanin	Depth of color								
Group A. Experimental biopsy taken 12 days after cessation of the treatment																			
1148	Black	+++	++++	D	0	0	0	++	++	++	++	++	++	++	++	++	++	0	
1155	Black	+++	++++	D	0	++	++	++	++	++	++	++	++	++	++	++	++	0	
1149	Black	Animal died during experiment																	
1145	Red	+++	++++	0	+++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
1152	Red	+++	++++	P	+++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
1153	Red	+++	++++	0	+++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Group B. Experimental biopsy taken 19 days after cessation of the treatment																			
1150	Black	++	++	D	0	0	0	++	++	++	++	++	++	++	++	++	++	0	
1154	Black	+++	++++	D	++	++	++	++	++	++	++	++	++	++	++	++	++	++	0
1156	Black	++	++	0	0	0	0	++	++	++	++	++	++	++	++	++	++	0	
1146	Red	0	++	P	0	0	0	++	++	++	++	++	++	++	++	++	++	0	
1147	Red	+++	++++	0	+++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
1151	Red	No melanocytes visible in control specimen. In the experimental specimen the melanocytes contained a small amount of melanin and possessed long thin complex dendritic processes.																	

The morphological appearances of the melanocytes in the experimental specimens from the black animals were very difficult to make out in detail, due to the large amount of free melanin present.

++++ = The greatest increase recorded  
 +++ = Very great increase  
 ++ = Great increase  
 + = Moderate increase  
 0 = Small increase

0 = No change  
 P = Change to peripheral concentration of melanin within melanocytes  
 D = Change to diffuse distribution of melanin within melanocytes  
 C = Change to intermittent aggregation of melanin within dendrites.

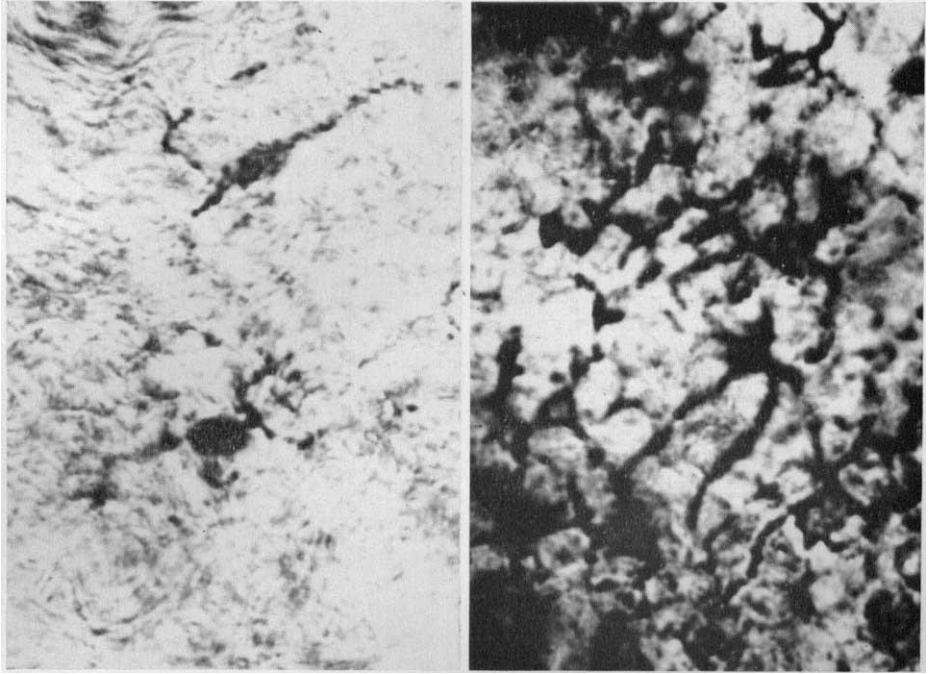


FIG. 3. Photomicrographs of skin sheets of anterior abdominal wall of red mature male guinea-pig (No. 1145); (a) before x-ray irradiation, (b) 12 days after x-ray irradiation. Shows a large number of melanocytes full of melanin and a considerable increase in the amount of free melanin present. Treated with Dopa reagent. No counterstain.  $\times 776$ .

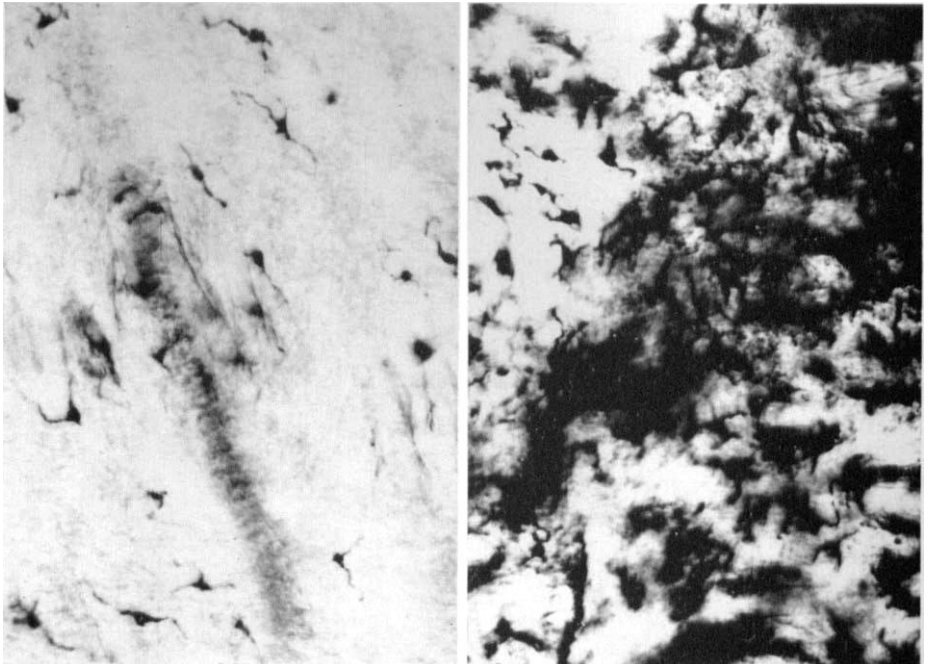


FIG. 4. Photomicrographs of skin sheets of anterior abdominal wall of red mature male guinea-pig (No. 1147); (a) before x-ray irradiation, (b) 19 days after x-ray irradiation. Shows a large number of melanocytes full of melanin. Note also the great increase in the amount of free melanin. Treated with Dopa reagent. No counterstain.  $\times 185$ .

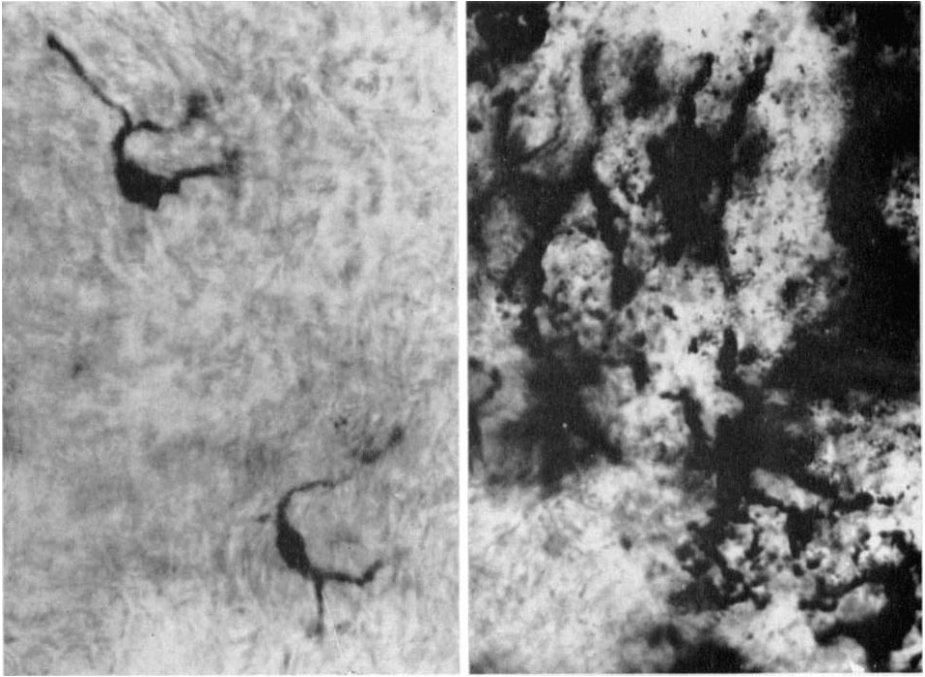


FIG. 5. High-power photomicrographs of skin sheets of anterior abdominal wall of red mature male guinea-pig (No. 1147) (compare with Fig. 4); (a) before x-ray irradiation, (b) after x-ray irradiation. Shows an increased number of melanocytes containing a very large amount of melanin. Note the increase in the length, width and complexity of the dendritic processes, and the considerable amount of free melanin present. Treated with Dopa reagent. No counterstain.  $\times 611$ .

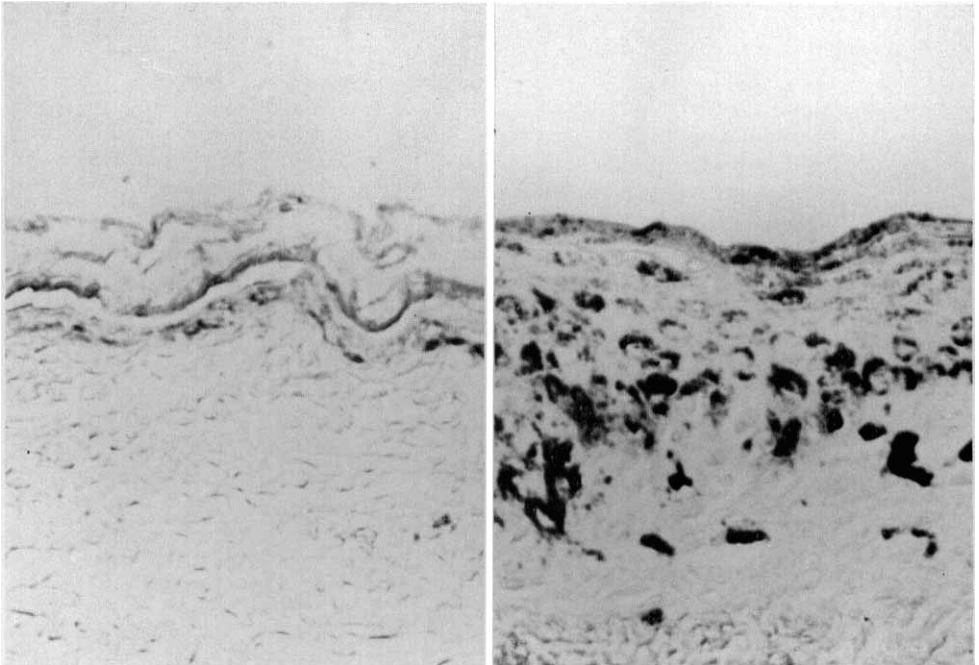


FIG. 6. Photomicrographs of vertical sections of anterior abdominal wall of black mature male guinea-pig (No. 1155); (a) before x-ray irradiation, (b) after x-ray irradiation. Shows a considerable increase in the amount of melanin present in all the layers of the epidermis, especially the basal layer. Note the melanophores filled with melanin in the dermis. Treated with Dopa reagent. Counterstained with weak eosin.  $\times 350$ .

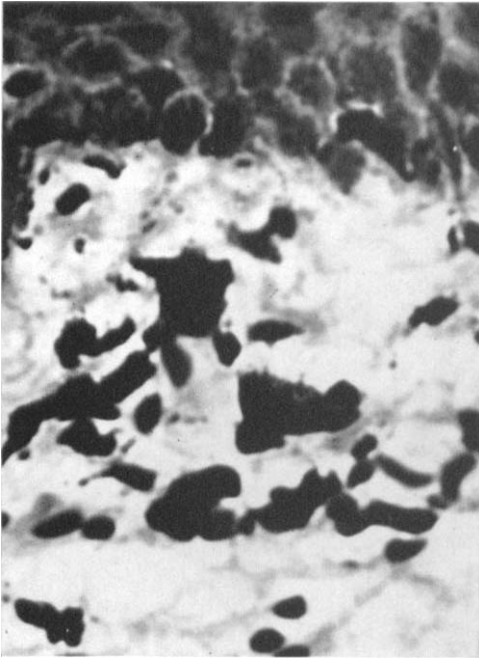


FIG. 7. Photomicrograph of vertical section of anterior abdominal wall of black mature male guinea-pig (No. 1156) after x-ray therapy. Shows a cluster of dermal melanophores filled with melanin. Treated with Dopa reagent. Counterstained with haematoxylin and eosin.  $\times 1756$ .

length, width and complexity of the dendritic processes.

The vertical sections showed an increase in the thickness of the epidermis in four of the animals, the increase mainly affecting the prickle cell layers. The "hill and valley" pattern of the dermo-epidermal junction appeared unchanged except in two animals (1155 and 1150) when it was slightly increased. A considerable rise in the amount of melanin was seen in all the layers of the epidermis. The melanocytes in the basal layer were very prominent having long, wide, branching dendritic processes filled with melanin (fig. 6). An occasional flattened, branched, pigment-containing cell could be seen lying superficial to the dermoepidermal junction, either among the prickle cells or very occasionally in the stratum corneum. A large number of dermal melanophores packed full of melanin granules were seen in all the experimental sections (fig. 7). A number of hair follicles had a degenerate appearance.

The melanocyte counts for the control and experimental skin specimens are shown in Table

II. In Group A, the increase in the amount of free melanin prevented accurate cell counting in two of the black animals, but the remaining three animals showed a marked rise in the melanocyte numbers. In Group B, the increased amount of free melanin again prevented accurate melanocyte counting in two of the black animals, but the remainder showed a considerable rise in the population density.

It was interesting to see that in the red guinea-pig No. 1151 the amount of melanin present within the control melanocytes was so small that it was impossible to identify the cells. However, following the x-ray irradiation, the cells filled with pigment and a count was easily made.

#### DISCUSSION

According to Warren (6), the first change noticeable in the skin after irradiation is a cessation of mitotic activity in the epithelial cells, followed in a few days by edema of the subcutaneous tissues with vaso-dilatation and evidence of vascular damage ranging from none to complete thrombosis. Later, the epithelium becomes thin

TABLE II

*Showing the effect of x-ray irradiation on the melanocyte counts in the skin of the anterior abdominal wall of the male guinea-pig*

No. of Animal	Color of Animal	Weight of Animal	Control	Experimental
A. Experimental specimen removed 12 days after cessation of treatment				
			<i>melanocytes per mm<sup>2</sup></i>	
1148	Black	720	671	*
1155	Black	425	685	*
1149	Black	460	726	Died
1145	Red	630	192	767
1152	Red	725	288	795
1153	Red	365	219	1192
B. Experimental specimen removed 19 days after cessation of treatment				
1150	Black	445	726	1041
1154	Black	485	822	*
1156	Black	535	753	*
1146	Red	645	219	671
1147	Red	695	178	479
1151	Red	510	0	301

\* Cells not countable owing to the presence of a very large amount of free melanin.

and the rete ridges tend to disappear, leaving an almost straight boundary between the epidermis and dermis with loss of the basement membrane. The skin appendages atrophy and disappear. There is an increased production of melanin by the "basal cells". Severe reactions may result in areas of depigmentation instead of hyperpigmentation (Saunders and Montgomery (7)).

A number of workers have studied the effect of thorium X (a radio-active substance emitting mainly alpha rays) on the process of pigmentation in human skin. Peck (8) was the first to investigate the pigmentary response by means of the dopa reaction. He described the metamorphosis of epidermal basal cells into "melanoblasts" and reported that at the height of the reactive process, practically every basal cell had become dendritic and contained Dopa oxidase. Becker, Fitzpatrick and Montgomery (9) repeated these experiments and found tyrosinase activity beginning three days after a five day exposure to an ointment containing 300 micro curies of thorium X per gram. Activity was seen to be restricted to specific dendritic cells, and no transformation of basal cells into melanocytes was observed. The latter were increased in number.

Chase and Smith (10) have found that x-ray irradiation may destroy melanocytes in the hair follicles of mice. Szabó (11) states that the same is true for the human epidermis where the majority of the melanocytes perish as a result of prolonged radiotherapy. He found that the surviving melanocytes enlarge and become hyperactive, while the epidermis becomes flattened and the majority of the hair follicles are destroyed.

Staricco (12) reviewed the findings of Peck and Becker *et al*, and carried out a study on the behavior of melanocytes stimulated with relatively small doses of thorium X. He believed that the previous workers had used too high concentrations of thorium X, which had complicated the picture by producing strong inflammatory changes in the skin and gross exfoliation of the epidermis. Two human subjects were used and the thorium X was painted on to the skin. From one of the subjects biopsy specimens were removed 24 hours, 7 days and 15 days after treatment, and from the second subject 7 days after treatment. The skin specimens were split and the dopa reaction performed on fresh epidermal

sheets, followed by fixation in formalin. His results showed that gross changes were much less severe than those described by Peck and Becker *et al*. The sheets of split skin showed dopa positive cells in all specimens; tyrosinase activity was found to be negative in normal skin and after 24 hours, but positive on the 7th and 15th days. On the 7th day, the melanocytes appeared more heavily pigmented, had larger and longer dendritic processes and larger cell bodies. The picture was similar after fifteen days. The number of melanocytes decreased slightly in the first subject at 7 days, and a further slight decrease occurred at 15 days. In the second subject an increase in the number of melanocytes occurred on the 7th day. This latter skin showed some acanthosis with elongation of the rete ridges. Examination of the separated epidermal sheets also revealed some dopa positive dendritic cells lying within the stratum spinosum and this was confirmed in the vertical sections. Melanophores were often seen in the upper dermis. Another finding of interest was that after 15 days many of the melanocytes appeared to have returned to their normal size. Staricco confirmed the work of Becker *et al* by finding that while the melanocytes contained more melanin granules after stimulation by thorium X, no increased pigmentation was seen in the neighboring basal cells.

The object of the present experiment in the guinea-pig was to study the effect of x-rays on melanogenesis using a dose which did not cause a visible erythema and thus produced the minimum of inflammatory changes. To anticipate the effect of radiation of a given quantity upon the skin is an exceedingly difficult problem. I was fortunate in having the co-operation and advice from Dr. van Miert, of the Department of Radiotherapy, who administered the treatment basing his calculations of dose on those administered to the human subject. In view of the fact that it is characteristic for biological effects following exposure to ionizing radiations to be subject to considerable delay, and for this delay to be greater when small doses are administered, it was decided to perform the second skin biopsy removal some time after the last treatment and to perform it at different times. (Group A twelve days after the last treatment; Group B nineteen days after the last treatment.)

The results showed that with the dose of x-rays used, melanogenesis was greatly stimulated. The

effect was seen to be slightly greater in the specimens removed 12 days after treatment than in those removed 19 days after treatment. The morphological changes were similar to those recorded by Becker *et al* (9) and Staricco (12). It was interesting to find that the number of melanocytes increased in the present experiment and this is in agreement with the findings of Becker *et al* (9). This increase could not be explained on the basis that the rete ridges had elongated, since this had only occurred to a minimal extent in two of the specimens. The rise in the count could, however, be explained on the basis that mitotic division of the melanocytes had taken place. On the other hand, it is possible that amelanotic dopa negative\* melanocytes are normally present in the basal layer of the epidermis, and these were stimulated into activity by the x-rays. In guinea-pig No. 1151, for example, no melanocytes were seen in the control specimen, but after x-ray irradiation large numbers were present.

The finding of a large increase in the amount of melanin within the cells of the different layers of the epidermis, including the basal layer, is not in agreement with the findings of Becker *et al* (9) and Staricco (12) for human skin, following thorium X treatment. This increased amount of intracellular melanin would suggest an increased cytotrine activity of the melanocytes following x-ray therapy. Staricco and Pinkus (13) were of the opinion that in human skin a temporary block occurs in pathological conditions which either does not permit the melanocytes to discharge their granules, or prevents the malpighian cells from accepting them.

The demonstration of high level pigmented dendritic cells in this experiment is in agreement with the findings of Staricco (12). Hendren and Pinkus (14) have shown that the epidermis exfoliates at a far greater rate after small doses of thorium X. This would suggest that following irradiation the upward movement of the melanocytes to the surface from the basal layer, which according to Billingham and Medawar (15) is their natural fate, is so rapid that they have insufficient time to discharge all their melanin and are thus found high up in the epidermis. It should be emphasized that only an occasional high level branched cell was observed in this experiment.

\* *i.e.* dopa negative under the conditions of fixation and incubation present in this experiment.

Dermal melanophores are rarely seen in sections of normal anterior abdominal wall skin of the guinea-pig. However, following x-ray therapy these greatly increase in number and are seen to be packed full of melanin granules. A similar picture was seen in the dermis following sodium hydroxide treatment (Snell (16)) and Staricco (12) reported an increase in number in human skin following thorium X therapy. The origin of the melanin is presumably from melanocytes and Malpighian cells which have undergone radiation injury. The melanin, having escaped from the damaged cells, passed through the dermo-epidermal junction to be phagocytosed by the dermal melanophores.

#### SUMMARY

1. The effect of x-rays on melanocytes and melanin in the skin of the anterior abdominal wall was studied in six pure black and six pure red male guinea-pigs.

2. The x-rays were administered at 50 K.V., 2 cms. F.S.D. with a surface dose of 500 rads. in 0.07 of a minute. The treatment was given in three sessions at weekly intervals and the experimental biopsy skin specimens were removed after an interval of 12 days and 19 days had elapsed following the last session.

3. The treatment resulted in a considerable increase in the amount of melanin present, both inside and outside the melanocytes. This was accompanied by an enlargement of the cell body size and an increase in the length, width and complexity of the dendritic processes. There was a marked increase in the melanocyte counts.

4. The increased melanogenesis was found to be slightly smaller in the skin specimens removed nineteen days after the last irradiation, compared with those removed twelve days after treatment.

5. The vertical sections showed a considerable rise in the amount of melanin present in all the layers of the epidermis. An occasional flattened pigment-containing high level branched cell was seen and a large number of dermal melanophores packed with melanin granules were noted following the treatment.

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