The Effect of Repeated Applications of Minute Quantities of Mustard Gas (\(\beta\beta'\)-Dichlorodiethylsulphide) on the Skin of Mice*

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INTRODUCTION

In earlier experiments, Fell and Allsopp (4) found that when low concentrations (0.05 mgm./cc.) of mustard gas are added to the nutritive medium of tissue cultures in vitro, the cells continue to divide but many do so abnormally forming multinucleate, hypertrophic and other atypical cells similar to those seen in malignant tumors.

In view of these observations it was decided to study the histological changes produced in the skin of mice by the repeated application of small doses of mustard gas with particular reference to the effect of such treatment on the epidermis (5).

MATERIAL AND METHODS

Two series of mice were used, the first (11 mice) being treated with a solution of 250 \(\gamma\) / cc. mustard gas in acetone and the second (10 mice) with a solution containing 50 \(\gamma\) / cc. mustard gas. The mice were painted about five times a week by dropping 0.05 cc. of the solution on the back with a calibrated pipette.

Two mice in each series died during the course of the experiment and, being unfit for histological study, were rejected. The rest died or were killed at different intervals (Table I) and the painted area of the skin was excised, fixed and sectioned. In most of the animals half the treated skin was fixed in acetic Zenker's fluid and sections were stained with azan, Ehrlich's hematoxylin and erythrosin or (series 1) Feulgen's method, while the other half was fixed in 80 per cent alcohol and stained by Gomori's method for the demonstration of alkaline phosphatase (9). The latter preparations were incubated for 20 to 24 hours in the glycerophosphate solution instead of the 2 hours recommended by Gomori, as previous experiments had shown that for scar tissue better results were obtained with the longer incubation (3, 6).

RESULTS

Series I

Observations on the living mice.—A few days after treatment had begun, the painted area became irritated. After 14 days large incrustations had developed. These were sloughed, carrying the hairs with them and leaving a smooth healed surface. By the end of the third week the skin was edematous and sometimes ulcerated.\(^1\)

By the 30th day the hair was growing again on the treated area which showed also ulcerating patches and sloughing keratin. Epilation was repeated about the 56th day but 16 days later the hair was again growing strongly. It was shed a third time about the 130th day and was not regenerated on the painted area until after treatment ended on the 271st day.

After the paintings ceased the skin began to heal and hair grew over the whole site after all the keratin had sloughed. This growth of hair, however, was thinner than usual and the painted area could easily be identified until the end of the experiment.

No sign of tumor formation was seen at any stage of the experiment.

Histological observations.—The skin fixed after 2 days' painting (Table I, No. 1) showed a very slight leukocytic infiltration of the dermis but no other change. That fixed after 32 days (No. 2) had a very thick keratin layer and an abnormally dense dermis with a fairly heavy leukocytic infiltration; unfortunately post-mortem changes prevented more detailed examination as the animal had died during the night.

The skin painted for 66 days (No. 3) varied in

\(^1\)When serious ulceration occurred, painting was suspended for 2 or 3 days.
structure in different regions. In places the epidermis had disappeared and was replaced by a thick scab, elsewhere it appeared attenuated and atrophic but in other parts it was greatly thickened and proliferated actively. The cells of the growing region looked almost normal but the hair follicles were very atypical and many were hyperkeratotic (as stated above, epilation had occurred a few days before). Ulceration had reached the fatty layer.

The painted skins of 4 animals killed on the 273rd day (Nos. 4-7) were very abnormal. In places the epithelium was greatly thickened (Fig. 3 were sharply defined and the cells fairly well differentiated. Many hair follicles were abnormal. As in No. 3, in some regions the epidermis seemed partly atrophied and consisted of a few enlarged cells only, while elsewhere it had been shed and replaced by a scab. Hyperkeratosis was seen in all four mice. The dermis was abnormally thick and dense beneath the actively proliferating epithelium and was composed mainly of young collagen fibers, but in the denuded areas there was very little fibrous material and ulceration extended to the fatty layer.

Part of the painted skin in each of these 4 mice had been treated by Gomori's method for the demonstration of alkaline phosphatase. The ulcerating areas differed in a curious way from ordinary wounds and heat burns in similar preparations. In the latter the scab appears nearly black and is always the most intensely reacting part of the section; this is due to its high content of polymorph leukocytes which contain much phosphatase (6). The scabs on the mustard-painted skins on the other hand gave almost no reaction although heavily infiltrated with polymorphs. The epidermis and superficial layers of the dermis were also nearly free of phosphatase but below this zone the dermis reacted strongly, although in uninjured skin it contains little or no phosphatase. In healing wounds and heat burns a strong reaction always accompanies the regeneration of collagen fibers (3, 6) and a similar correlation between fiber-formation and phosphatase activity seemed to exist in the mustard gas lesions.

<table>
<thead>
<tr>
<th>Series</th>
<th>Exp. no. of mouse</th>
<th>Duration of painting</th>
<th>Approx. total dose of painting</th>
<th>Survival after end of painting</th>
<th>Total period of survival from beginning of painting</th>
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<tr>
<td>1</td>
<td>1</td>
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<td>25γ</td>
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1) and contained many atypical cells similar to those present in cultures grown in medium containing low concentrations of mustard gas (4). Multinucleate cells (Fig. 2) with nuclei of widely varying size were very common and all stages in their development from abnormal mitosis were seen. Hypertrophic nuclei (Fig. 3) and nuclei with enormously enlarged plasmasomes were also numerous. A large proportion of the mitotic figures were abnormal; clumping of the chromosomes, chromosome lag (Fig. 4) and more rarely polyploid cells (Fig. 5) and multipolar division were encountered. Somewhat similar abnormalities were observed by Gillette and Bodenstein in tadpoles grown in solutions of the "nitrogen mustard," methyl-bis-(β-chlorethyl)-amine (7), and by Koller, Ansari and Robson (10, 11) in the mitotic cells of Tradescantia treated with mustard gas. In 3 mice there were areas of active epithelial downgrowth into the dermis, but these downgrowths were sharply defined and the cells fairly well differentiated. Many hair follicles were abnormal. As in No. 3, in some regions the epidermis seemed partly atrophied and consisted of a few enlarged cells only, while elsewhere it had been shed and replaced by a scab. Hyperkeratosis was seen in all four mice. The dermis was abnormally thick and dense beneath the actively proliferating epithelium and was composed mainly of young collagen fibers, but in the denuded areas there was very little fibrous material and ulceration extended to the fatty layer.

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Healing was well advanced in a painted area fixed 79 days after the end of treatment (No. 8). The epidermis was still slightly thickened, though no abnormal cells and very little mitosis were seen, and many of the hair follicles were cystic. The dermis was rather more dense than usual but the strong phosphatase reaction seen at the previous stage had nearly disappeared, as in a fully healed wound.

In a mouse killed 100 days after the end of painting (No. 9) there was a slight local thickening of the epidermis, some hyperkeratosis and a reduction in the normal number of hair follicles. The dermis seemed normal and gave little phosphatase reaction.

Series 2

Observations on the living mice.—Epilation first appeared rather later than in Series 1, i.e., after 3 weeks' painting. Hair began to regenerate about the 38th day and was again being shed by the 62nd day. The treated skin was ulcerated much less than in Series 1, but appeared to be irritable. There was no actual tumor formation. In the earlier stages, 2 or 3 mice developed thickenings which resembled incipient tumors, but these regressed and were probably areas of hyperkeratosis.

Histological observations.—The epidermis of the mice examined during (No. 1) and two days after (Nos. 2-5) painting was abnormally thick but proliferation was much less active than in the equivalent mice of Series 1. The cells were somewhat enlarged and those of the basal layer, which showed very little mitosis, were mostly transformed into prickle cells. Abnormal mitotic figures and multinucleate cells occurred but were much less numerous than in Series 1. The hair follicles were usually hyperkeratotic or cystic. The dermis contained many fine, newly formed collagen fibers and was fairly heavily infiltrated with leukocytes.

The distribution of phosphatase resembled that in the skin of Series 1, i.e., the reaction was most intense in the regenerating fibrous tissue and the proximal ends of the hair follicles, while the scabs and the most superficial part of the tissue contained very little phosphatase.

Discussion

The results of our experiments have shown that the repeated application of small doses of mustard gas over long periods has a very pronounced effect on the epidermal nuclei, the cytological abnormalities produced resembling those previously seen in fibroblasts cultivated in medium containing low concentrations of mustard gas (4). On the other hand, differentiation is not affected in the regenerating epidermis, a result which agrees with that of Gillette and Bodenstein (7) who found that in tadpoles treated with a nitrogen mustard compound "the agent selectively affects mitosis and not differentiation."

It is interesting to compare the histological changes in the skin of our experimental animals with those appearing in mice treated with 3,4-benzpyrene (8). Both treatments produced a cumulative effect, an alternation of degeneration and repair indicated macroscopically by alternating epilation and regeneration of hair, and nuclear disturbances manifested in abnormal mitosis, multinucleate cells, hypertrophic nuclei, etc.

There are, however, important differences between the effects of the two substances. Painting with dilute mustard gas has a more drastic nuclear action than painting with benzpyrene and causes a much larger proportion of severe cell abnormalities. In the skins treated with mustard gas, the hyperplastic regions are usually near an ulcerating area and the cell proliferation appears to be no greater than is required for the replacement of the damaged tissue. When epithelial downgrowth into the dermis occurs, the downgrowing processes have a sharply defined outline and do not show that excessive proliferation of the undifferentiated basal cells followed by diffuse invasion of the surround-}

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mice but does not interfere with differentiation or cause tumors. These observations further emphasize the fact that although abnormal mitosis is a usual feature of malignant tissue it is not a specific character of malignancy.

The effect of repeated applications of dilute mustard gas on the alkaline phosphatase of the skin is interesting. The mustard gas appears to destroy or inactivate the phosphatase in the scab, which in wounds contains a large concentration of the enzyme, but this inhibitory effect does not seem to penetrate very deeply as the regenerating fibrous tissue of the dermis reacts strongly as it does in a healing wound or heat burn.

SUMMARY

1. Experiments were made to investigate the histological effect on the skin of mice of the repeated application of small doses of mustard gas (12.5 \( \gamma \) and 2.5 \( \gamma \)).

2. The following similarities between skin treated with small doses of mustard gas and that treated with 3,4-benzpyrene were observed: the effect of the treatment was cumulative; there was a repeated alternation of degeneration and repair; nuclear abnormalities (abnormal mitosis, multinucleate cells, hypertrophic nuclei, etc.) were produced in the epidermis; in certain areas the epidermis became hyperplastic.

3. The effect of small doses of mustard gas differed from that of 3,4-benzpyrene as follows: no tumors developed under the conditions of our experiments; the nuclear disturbance was more drastic causing a much greater proportion of cell abnormalities; the hyperplastic regions of the epidermis were much more differentiated and showed no sign of the diffuse invasion of the connective tissue which characterizes malignancy; cell proliferation appeared to be no greater than was required to replace the damaged tissue.

4. The effect resembled that of a single massive dose of x-rays.

5. Preparations of the skins treated with mustard gas were made by Gomori’s method for the demonstration of alkaline phosphatase. The scab and immediately subjacent tissue gave no reaction, but the regenerating dermal tissue reacted strongly (in heat burns the scab reacts even more intensely than the regenerating connective tissue; the normal dermis has little or no phosphatase).

6. The treated areas had largely recovered in mice examined 65 to 100 days after cessation of painting, but the skin was not quite normal.

REFERENCES


DESCRIPTION OF FIGURES 1 TO 5

The photomicrographs were made by Mr. V. C. Norfield, Head Assistant at the Strangeways Research Laboratory.

Fig. 1.—Section of the skin of a mouse from Series 1 killed on the 273rd day (i.e., 2 days after the end of treatment) showing the greatly thickened epidermis adjacent to an ulcerating area. Hematoxylin and erithrosin stain. Mag. \( \times \) 40.

Fig. 2.—Section of the skin of the same mouse showing a multinucleate cell and several hypertrophic cells in the epidermis. Feulgen stain. Mag. \( \times \) 900.

Fig. 3.—Section of the skin of another mouse from the same series killed on the 273rd day showing a hypertrophic epidermal cell. Feulgen stain. Mag. \( \times \) 900.

Fig. 4.—An abnormal telophase with chromosome ‘lag’ in the same skin as that shown in Fig. 3. Feulgen stain. Mag. \( \times \) 900.

Fig. 5.—Polypoid cells in the same skin. A multinucleate cell is also seen. Feulgen stain. Mag. \( \times \) 900.
Figs. 1–5
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