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 7/ cc. mustard gas in acetone and the second (10 mice) with a solution containing 50 7/ 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.

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Note: Because of accelerated production schedule, author has not read proof of this paper.
structure in different regions. In places the
ermis 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 grow-
ing region looked almost normal but the hair fol-
llices were very atypical and many were hyperker-
rotic (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.
1) and contained many atypical cells similar to
those present in cultures grown in medium con-
taining 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 encoun-
tered. Somewhat similar abnormalities were ob-
served by Gillette and Bodenstein in tadpoles
grown in solutions of the “nitrogen mustard,”
methyl-bis-β-chlorethyl)-amine (7), and by Kol-
ner, Ansari and Robson (10, 11) in the mitotic
cells of Tradescantia treated with mustard gas. In
3 mice there were areas of active epithelial down-
growth into the dermis, but these downgrowths
were sharply defined and the cells fairly well differ-
entiated. 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 re-
placed 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

<table>
<thead>
<tr>
<th>Series</th>
<th>Exp. no. of mouse</th>
<th>Duration of painting (from beginning of painting)</th>
<th>Approx. total dose of painting</th>
<th>Survival after end of painting</th>
<th>Total period of survival</th>
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<tbody>
<tr>
<td>1</td>
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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 in places, 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 superfiacial 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-benz-pyrene (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 surrounding tissue which characterizes skins treated with benzpyrene. The fundamental difference between the histological effects of treatment with dilute mustard gas and with benzpyrene seems to be that while the former disturbs mitosis but permits differentiation, the latter stimulates mitosis but inhibits differentiation.

This difference may explain why, under the conditions of our experiments, no tumors were formed in response to the mustard gas. Many more animals would have to be treated before the non-carcinogenicity of the agent could be finally established, but our results agree with those of Berenblum (1, 2). Working with experimental conditions that differed from ours in some respects, he not only obtained no tumors in mice by the repeated application to the skin of small quantities of mustard gas, but also made the important observation that similar amounts of the agent inhibit the carcinogenic action of tar on the skin.

There is an interesting similarity between the histological effects of the repeated application of small quantities of mustard gas and of a single massive dose of x-rays. Glucksmann (unpublished results) has recently shown that such an irradiation produces mitotic abnormalities in the skin of
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 y and 2.5 y).

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 Gömörí’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**


Figs. 1–5
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