Localized Changes in Methylcholanthrene-Treated Epidermis*

Hsu-mu Liang**M.S.
(Department of Anatomy, Washington University School of Medicine, and Barnard Free Skin and Cancer Hospital, St. Louis 3, Missouri)

Received for publication August 24, 1947)

Since Yamagiwa and Itsikawa in 1915 (14) succeeded in inducing cancer in rabbits by means of coal tar, investigators have been aware that the induced cancers appear within fairly sharply defined foci. Most data secured by chemical analysis do not supply direct information about these foci; because, in order to obtain enough material for analysis the treated epidermises of several mice are usually pooled so that with the foci is included a large volume of epidermis that does not undergo local malignant transformation. Methods are needed that will reveal the chemical composition of areas of tissue of microscopic size. The immediate objective of this investigation is to learn more about these focal tissue changes by microscopic examination of whole mounts of epidermis—a technic more effective for this purpose than the study of stained sections. Ultimately it is hoped that the histochemical method can be applied to such whole mounts.

MATERIAL AND METHODS

The backs of young female Swiss mice 6 to 8 weeks old were shaved, care being taken to avoid mechanical injuries. Three days later 0.6 per cent methylcholanthrene in benzene was applied to the shaved areas with a camel’s hair brush regularly at 10 A.M. (St. Louis time) because the mitotic frequency of mouse epidermis is highest at this time (4). Only three paintings were made. A second group of mice were similarly treated with benzene only. A third group was examined untreated.

Mice of the first 2 groups were sacrificed for examination 7 to 30 days after the first painting by a single blow on the head at 10 A.M. The painted area of skin was in each case removed and spread over a piece of filter paper. Both skin and paper were immersed in a jar of 1 per cent acetic acid (5) and were maintained at 5 °C. for 3 to 5 hours in order to macerate the connective tissue fibers which bind the epidermis to the dermis. Maceration in excess of 5 hours damages the basal layer and greatly impairs stainability of the cells. After 3 to 5 hours in cold acetic acid the skin was washed in distilled water at room temperature. It was then stretched moderately over a piece of cork board immersed in a shallow dish of distilled water and the epidermis was gently separated from the dermis using an iridectomy knife or a spear point dissecting needle.

The sheets of epidermis were stained with a high contrast hematoxylin (1/2 stock solution of Harris hematoxylin and 1/2 sat. aq. sol. of aluminum ammonium sulfate) for 15 minutes (3). They were then thoroughly washed with several changes of distilled water until all excess stain was removed (no acid or ammonia bath being necessary), dehydrated in alcohol, cleared in cedar wood oil, rinsed in xylol and mounted in clarite with the basal epidermal layer uppermost.

OBSERVATIONS

Normal epidermis and hair follicles.—Fig. 1 illustrates a whole mount of normal epidermis. This is generally traversed by fine and coarse furrows which form a close network. The meshes of the network formed by the coarse furrows tend to be scale-like in shape, whereas those of the fine furrows are polygonal or trapezoidal in shape. The separated epidermis is composed of 3 to 5 layers of cells in which cornified, granular and basal layers can be recognized. The cornified layer is 1 to 2 cells thick and composed of thin, clear, dead and scale-like cells. The granular layer consists of 1 to 2 layers of flattened hexagonal or rhombic cells. Their nuclei are large oval or spherical in shape and their cytoplasm contains fine granules of fairly uniform size which stain faintly in hematoxylin. A few binucleated cells of similar characteristics occur in this layer. The basal layer consists of only 1 layer of cells. These are polygonal in shape with deeply chromatic spherical or oval nuclei which are only about 1/2 the size of those of the granular cells. Cells with clear cytoplasm and darkly stained nuclei are very often found intermingled with the basal cells. In addition, darkly stained multipolar and spindle-shaped cells are

* A part of a dissertation submitted to the Board of Graduate Studies of Washington University in partial fulfillment of the requirements for the degree of Master of Science in Anatomy.
** Fellow of American Bureau for Medical Aid to China.
† Aided by grants from the U. S. Public Health Service, the Charles F. Kettering Foundation and the Women’s Advertising Club of St. Louis.
found scattered among the basal cells or aggregated near large hair follicles (Fig. 3). The shape of the nuclei and of the processes of these cells indicate that they are probably of the same type as those described by Langerhans and as "tactile cells" by Merkel and Ranvier (9, 10). Their origin and function remain unknown.

Hair follicles of normal specimens are better seen in whole mounts of dermis from which epidermis has been removed. Fig. 2 represents such a preparation from the back of a mouse. It shows two types of hair follicles which differ both in length and diameter. The smaller ones are present in much greater numbers and usually 3 to 5 are arranged in a row. The larger follicles are wider and about twice as long as the smaller ones. They are scattered singly among the small follicles. One is seen near the middle of the lower part of Fig. 2. They can also be recognized in whole mounts of epidermis because they are often accompanied by groups of the darkly stained multipolar and spindle shaped cells already referred to. Large follicles marked by such groups are illustrated in Figs. 1 and 3.

Epidermis and hair follicles treated with methylcholanthrene.—In general the changes observed agree very well with reports published by various authors, and, particularly Cramer (6, 7). But use of the whole mount technic has revealed many additional details of local changes both in epidermal cells and hair follicles.

Marked localized changes were observed in the painted epidermal cells. Such changes occurred in 1 to 5 irregularly spaced foci. The earliest local alterations were usually located at the junctions of the hair follicles and the basal epidermal cell layer but also appeared in a follicular region (Fig. 5). They were composed of groups of cells either closely packed together or rather loosely dispersed. Quite often leukocytes and ring cells (metamyelocytes) were seen in these areas. Areas of local increase in cell number were recognized as early as 7 days after painting. In some instances there were indications of the spreading of these cells (Fig. 6).

Marked cell multiplication within the affected area resulted in cells streaming out from the center and not only disrupting the pattern of regenerating hair follicles but also pushing the follicles away from the center of activity. This is shown in Fig. 7. The extensive peripheral movement of hair follicles eventually caused the erosion of the central core of the affected area which in turn was accompanied by the appearance of opaque-looking cells with pycnotic nuclei, leukocytic infiltration, and finally by ulceration of the central focus (Fig. 8).

Follicular response was less localized than that of the interfollicular epidermis. Degenerated, enlarged and fused follicles were observed. The degenerated follicles varied slightly in shape and size and somewhat resembled filliform papillae of the tongue. Most of the follicles were conically shaped with sharply pointed tips, though some appeared to be club shaped with blunt or slightly enlarged tips. They were composed of small and darkly stained cells (Fig. 9). Enlarged follicles were balloon-like with or without conical tips (Fig. 9). The cells appeared to be of three different types: long spindle shaped cells surrounding the junctional line between the follicle and basal epidermal layer, flattened cells with clear cytoplasm and faintly stained round nuclei forming the main part of the follicle and small darkly stained cells, similar to those in degenerated follicles, located at the ends of conical tips. Fused follicles were probably formed by merging of several follicles (Fig. 10). Usually one of the member follicles was much larger than the others. The same cell types were formed as in enlarged follicles. In one specimen a highly localized response involving only a single hair follicle was observed. (Fig. 12).

Variation in response.—Considerable individual variation in response of animals, even of the same species, strain, age and sex, to chemical carcinogens has been noted by many workers (7, 12) and has been observed in this study. This is particularly true in the case of the follicular response. The several different types of abnormal follicles were seldom found in one animal. Figs. 9, 10, and 12 are of specimens taken from 3 animals each exhibiting a different type of change in the follicles. Fig. 9 shows degenerated and enlarged follicles, Fig. 10 fused follicles and Fig. 12, a single enlarged follicle.

Differences in rate of development of the various induced alterations also were observed. In more susceptible animals marked local alterations were found as early as 7 days after the first painting. In other animals as much as 20 days were required to produce comparable effects. Early signs of progressive alterations were most frequently observed from 15 to 20 days after the first painting. From 20 days onward, the epithelial cells as well as the general pattern of the epidermis of less affected areas tended to revert to the normal pattern. (Fig. 11). It seems, therefore, that a peak of response was reached at approximately the 15th day.

Benzene control.—The effect of benzene on epidermal cells has been described variously by many authors. Page (11) reported that no significant cyto-
logical change in the skin was caused by benzene, but Pullinger (12) pointed out that benzene does cause severe damage followed by healing with hyperplasia and hyperkeratosis. Cramer and Stowell (7, 13) found similar effects of benzene on epidermis. However, all appear to agree that the action of benzene alone is very different from that of benzene plus carcinogen. The benzene control group in this study showed characteristic epidermal modifications. Fig. 4 illustrates the condition of the epidermis 15 days after the first painting with benzene and should be compared with Fig. 1 showing normal epidermis. Note in Fig. 4 a slight degree of hyperplasia; disappearance of fine furrows; the cluster of cells accompanying the large hair follicles is more dispersed and the hair follicles are considerably enlarged.

DISCUSSION

Localized carcinogenic changes can be expected because carcinomas always appear in restricted areas. Cramer (7) noticed that “an area of skin exposed to the action of a carcinogen over a prolonged period does not react equally to the carcinogen but shows considerable variation in the degree of response. The final carcinogenic response is restricted to one or sometimes several localized centers.”

The present results indicate that localized changes are present in epidermal cells as well as the follicular cells. The localized changes shown by the epidermal cells are closely confined to discrete areas. They seem to consist of rapidly multiplying cells with progressive elimination of hair follicles and subsequent erosion of the central area.

Ulcerations were observed in mice treated under similar condition by Cramer (7), but he did not trace their development. It is commonly believed that most carcinomas develop without previous ulceration, yet it seems logical that ulcerations, developed in this way from the centers of localized changes, may be the precursors of malignancy. The presence of such locally changed areas, which can be recognized with the naked eye after removal of the skin and the ease of their separation from the remainder of the epidermis, may possibly open the way for histochemical analysis of actually precancerous epidermis.

It is not surprising to note that the earliest local changes in the epidermis originate most commonly near hair follicles since, under normal conditions, hair follicles are more active in mitosis (7) and after chemical or mechanical injury play a prominent part in regeneration (1, 2, 8).

SUMMARY

Normal, benzene- and 0.6 per cent methylcholanthrene-painted epidermises were studied in 80 young Swiss female mice by means of the whole mount technic. The epidermis was separated from the dermis by cold acetic acid, stained with hematoxylin and mounted with the dermal surface up.

Microscopic changes were examined up to 30 days after the first painting. Despite individual variations, definite localized epidermal and follicular alterations were observed in methylcholanthrene-painted epidermis. The first indication of a local epidermal change was the presence of cell clusters in a radiating pattern which usually appeared at the junction of hair follicles and the basal epidermal layer. Extensive multiplication of these cells caused disruption of the follicular pattern and eventual ulceration in the center of the proliferating area.

Follicular responses were less localized than those of epidermal cells. Several types of changes such as degenerated, enlarged and fused follicles were observed.

ACKNOWLEDGMENT

The author wishes to express his grateful thanks to Professors E. V. Cowdry and A. I. Lansing for their advice and criticism throughout the study; and to Mr. M. W. Rhoades for the photomicrographs.

REFERENCES


DESCRIPTION OF FIGURES 1 TO 4

All are whole mount preparations, stained with hematoxylin.

Fig. 1.—Normal epidermis, showing fine and coarse furrows which form networks; the broken ends of hair follicles arranged in rows; the locations of two large follicles, each of them accompanied by a group of darkly stained cells. Mag. × 80.

Fig. 2.—Normal dermis, viewed from the subcutis. Small hair follicles and two large follicles, one at the middle of top and bottom of the photograph. Mag. × 80.

Fig. 3.—Normal epidermis. Darkly stained multipolar and spindle-shaped cells aggregated at the location of a large follicle and scattered among basal cells. Mag. × 340.

Fig. 4.—Benzene treated epidermis, 15 days after the first painting. Disappearance of fine furrows, slight enlargement of hair follicles, a more dispersed arrangement of the multipolar and spindle shaped cells at the sites of two large follicles. Mag. × 80.
DESCRIPTION OF FIGURES 5 TO 8

All are whole mounts of methylcholanthrene-treated epi-
dermis, stained with hematoxylin, all photographs Mag. 
× 80, showing localized changes of epidermal cells and 
some follicular changes.

Fig. 5.—Ten days after first painting. Early local 
changes.

Fig. 6.—Twenty days after first painting. Cells spread 
out from the changed area.

Fig. 7.—Fifteen days after first painting. Cell multipli-
cation pushing follicles away from the center.

Fig. 8.—Fifteen days after first painting. Extensive 
peripheral shift of follicles and ulceration of the central 
tissue.
DESCRIPTION OF FIGURES 9 TO 12

All are whole mounts of methylcholanthrene-treated epidermis, stained with hematoxylin, showing mainly localized follicular changes. All photographs Mag. X 80.

Fig. 9.—Twenty days after first painting. Filamentous atrophied follicles and balloon-like enlarged follicles.

Fig. 10.—Twenty days after first painting. Fused follicles.

Fig. 11.—Thirty days after first painting, less affected area.

Fig. 12.—Twenty days after first painting. A single enlarged follicle.
Localized Changes in Methylcholanthrene-Treated Epidermis

Hsu-mu Liang