INSTITUTE REPORT NO. 202

HISTOLOGIC CHANGES CAUSED BY APPLICATION OF LEWISITE ANALOGS TO MOUSE SKIN AND HUMAN SKIN XENOGRAFTS

EVELYN L. McGOWN, PhD
THEODORE van RAVENSWAAY, MD, LTC MC USAR
CECILIO R. DAMLAO, BS
RICHARD J. O'CONNOR, MS
and
KENNETH E. BLACK, MD, COL MC

DIVISION OF RESEARCH SUPPORT
and
DIVISION OF CUTANEOUS HAZARDS

JUNE 1985

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Histologic changes caused by the application of lewisite analogs to mouse skin and human skin xenografts--McGown et al

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER Institute Report No. 202	2. GOVT ACCESSION NO. AD-A159	3. RECIPIENT'S CATALOG NUMBER 554
4. TITLE (and Subtitle) Histologic Changes Caused by Application of Lewisite Analogs to Mouse Skin and Human Skin Xenografts.		5. TYPE OF REPORT & PERIOD COVERED Final 1 May 83 - 30 Nov 84 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Evelyn L. McGown, PhD Theodore van Ravenswaay, MD,LTC,MC,USAR Cecilio R. Dumlao, BS, SP5, Richard J.O'Connor, MS Kenneth E. Black, MD, COL, MC		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS Division of Research Support Letterman Army Institute of Research Presidio of San Francisco, CA 94129-6800		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS Proj #3M162734A875 Task #BD, Work unit #306
11. CONTROLLING OFFICE NAME AND ADDRESS U.S Army Medical Research & Development Command Fort Detrick Frederick MD 21701-5010		12. REPORT DATE 1985 13. NUMBER OF PAGES 35
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) UNCLASSIFIED 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

THIS DOCUMENT IS APPROVED FOR PUBLIC RELEASE AND SALE: ITS DISTRIBUTION IS UNLIMITED.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Nude mouse; Human Skin Xenografts; Arsenical; Vesicant; Morphology; Lewisite analogs

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Phenyldichloroarsine (PDA), a vesicating analog of lewisite, was applied in an ethanol carrier to human skin xenografts on nude mice and directly to the ungrafted nude mouse skin. Controls areas received ethanol. ~~The animals were~~ killed from 30 min to 48 hr after application of 1.26 umoles PDA onto ~~an area of 0.126 cm^2~~. Skin sections were examined by light and electron microscopy. Under light microscopy, we observed the following changes in PDA-treated human

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

skin grafts: 1) epidermal cellular nuclear degeneration (apparent by 4 hr with increasing severity through 48 hr); 2) loss of epidermal cytoplasmic basophilia (apparent by 4 hr, maximum within 12 hr); 3) epidermal cytoplasmic vacuolization (vacuoles appeared within 4 hr and increased in size through 24 hr); 4) cleft formation within the basement membrane zone (apparent by 12 hr, increasing in severity through 24 hr); 5) inflammation [polymorphonuclear leukocyte (PMN) infiltration], apparent by 4 hr and increasing through 48 hr. The PMNs formed a wall around the lesion, but did not infiltrate the treated area. In nude mouse skin, the changes were similar and occurred more quickly, except for cytoplasmic vacuoles which were only occasionally observed. Nude mouse hair follicles and sebaceous glands showed similar cellular changes at approximately the same time as did epidermal cells. Transmission electron microscopy of mouse skin exposed to PDA up to 6 hr revealed a widening of intercellular spaces with attenuation of desmosomes. The subepidermal clefts resulted from separation within the lamina lucida with the lamina densa forming the base of the cleft.

The following additional lewisite analogs were applied in ethanol carriers to nude mouse skin: phenylarsine oxide, phenydiiodoarsine, (trans) chlorovinylarsine oxide and (trans) chlorovinyldiiodide. The lesions caused by these analogs were reproducible and histologically indistinguishable from that cause by exposure to PDA. The identities of the molecular lesions and the locations of the arsenical-sensitive sites are unknown.

ABSTRACT

Phenyldichloroarsine (PDA), a vesicating analog of lewisite, was applied in an ethanol carrier to human skin xenografts on nude mice and directly to the ungrafted nude mouse skin. Control areas received ethanol. The animals were killed from 30 min to 48 hr after application of 1.26 umoles PDA onto an area of 0.126 cm^2. Skin sections were examined by light and electron microscopy. Under light microscopy, we observed the following changes in PDA-treated human skin grafts: 1) epidermal cellular nuclear degeneration (apparent by 4 hr with increasing severity through 48 hr); 2) loss of epidermal cytoplasmic basophilia (apparent by 4 hr, maximum within 12 hr); 3) epidermal cytoplasmic vacuolization (vacuoles appeared within 4 hr and increased in size through 24 hr); 4) cleft formation within the basement membrane zone (apparent by 12 hr, increasing in severity through 24 hr); 5) inflammation [polymorphonuclear leukocyte (PMN) infiltration], apparent by 4 hr and increasing through 48 hr. The PMNs formed a wall around the lesion, but did not infiltrate the treated area. In nude mouse skin, the changes were similar and occurred more quickly, except for cytoplasmic vacuoles which were only occasionally observed. Nude mouse hair follicles and sebaceous glands showed similar cellular changes at approximately the same time as did epidermal cells. Transmission electron microscopy of mouse skin exposed to PDA up to 6 hr revealed a widening of intercellular spaces with attenuation of desmosomes. The subepidermal clefts resulted from separation within the lamina lucida with the lamina densa forming the base of the cleft.

The following additional lewisite analogs were applied in ethanol carriers to nude mouse skin: phenylarsine oxide, phenyldiiodoarsine, (trans)chlorovinylarsine oxide and (trans)chlorovinyldiiodoarsine. The lesions caused by these analogs were reproducible and histologically indistinguishable from that caused by exposure to PDA. The identities of the molecular lesions and the locations of the arsenical-sensitive sites are unknown.

PREFACE

SP4 J Newland, SP5 K Freda, SP5 A Somera and Ms L Cote provided excellent technical assistance. Lottie Applewhite, LAIR editor, provided numerous editorial improvements.

TABLE OF CONTENTS

Histologic Changes Caused by Application of Lewisite Analogs to Mouse Skin and Human Skin Xenografts--McGown et al

The skin vesicating action of organic arsenicals is well known (1,2), and yet their mechanism of action is poorly understood. Thiols are the biochemical entities most sensitive to arsenicals (3). Because of the ubiquity of biological sulfhydryls, many enzymes and other proteins could be involved in arsenical toxicity. Ketoacid oxidases comprise the most sensitive class of enzymes reported to date, due to their dithiol-containing lipoic acid cofactor. The rise in blood ketoacids and depressed glucose utilization during acute arsenical poisoning suggests that ketoacid oxidases are involved in systemic arsenical toxicity (3). However, there is no direct evidence that inhibition of these enzymes is involved in skin vesication and injury.

The design of antidotes to vesicant arsenicals requires a knowledge of their molecular mechanisms and sites of actions. Because there is no ethically acceptable means of studying the effects of these toxic substances on the skin of human volunteers, an appropriate in vitro system or animal model is needed. One such model is the human skin grafted athymic nude mouse (4,5). This animal model was recently established at LAIR. Phenyldichloroarsine (PDA) is an analog of lewisite with similar vesicant potency (1,2). The study described in this report was intended to determine the morphological changes in nude mouse skin and human skin xenografts on the nude mouse following application of PDA.

METHODS

Phenyldichloroarsine (PDA) was purchased from Research Organic/Inorganic Chemical Corp., Sun Valley, CA. It was purified by repeated distillation at 128°C under 15 mm Hg of nitrogen and the purity (> 99%) was verified by infrared spectrophotometry. Phenyldiiodoarsine was prepared from PDA by direct halogen exchange. This involved reaction of PDA with a ten-fold excess of potassium iodide, followed by solvent extraction, filtration and vacuum removal of the solvent. (Trans)chlorovinyldiiodoarsine and (trans)chlorovinylarsine oxide were obtained through the courtesy of

S. Hallowell; Chemical Research and Development Center; Aberdeen Proving Ground, MD. (We refer to these compounds as lewisite iodide and lewisite oxide.) Phenylarsine oxide was purchased from ICN-K&K Laboratories, Inc.; Plainview, NY. Immediately before use, PDA and lewisite iodide were dissolved in anhydrous ethanol to a concentration of 1.25 M. Preparation of 1.25 M solutions of phenylarsine oxide and lewisite oxide was accomplished by addition of 2.5 M HCl. Phenyldiiodoarsine was prepared to 0.25 M because of its lower solubility in ethanol.

Six-week-old athymic nude mice were purchased from Harlan Industries, Indianapolis, IN. Skin specimens (post-rhytidectomy) were obtained from volunteers as a by-product of surgery at the Letterman Army Medical Center (LAMC) under the approved LAMC Protocol Addendum CH-81-01, "Human Skin for Purposes of Research Concerning Penetration of Chemicals and for Studies of Function." Skin grafting was done according to the standard operating procedure described in LAIR OP-DCH-37. Whenever an experiment used grafts which had been obtained from more than one donor, the animals corresponding to each donor were distributed among the treatments groups such that all groups contained grafts from all donors, if possible. The mice were anesthetized with an intraperitoneal injection of chloral hydrate (0.56 mg/g of body wt) and treated in a fume hood.

PDA was applied topically to 38 grafted and 44 ungrafted mice. One microliter of 1.25 M PDA in ethanol was placed with a micropipet onto a 0.125 cm^2 circular area of nude mouse skin or human skin graft and one microliter of ethanol was placed onto a separate area as a control. After 0.5 to 48 hr, they were killed by cervical spine dislocation. The treated areas of skin were excised, fixed in 10% neutral buffered formalin, embedded in paraffin for sectioning and stained with hematoxylin and eosin. Samples intended for electron microscopy were fixed in Karnovsky's fixative and embedded in EPON resin. To minimize subjective bias during review, all light microscopy slides were coded and randomized. To evaluate the development of the lesion as a function of time, we devised a semi-quantitative system to score the extent of damage (localized to widespread) or degree of damage (none to marked) in the most severely affected area of the lesion. Nuclear degeneration was scored 0 to 4 as follows: 0 (no change), 1 (detectable pyknosis), 2 (moderate pyknosis), 3 (loss of basophilia with nuclear outline remaining), and 4 (complete loss of nuclei). To evaluate changes in cytoplasmic staining, the sections were examined under low power (4X) magnification. The extent of loss of cytoplasmic basophilia in 3 contiguous low power fields (LPF) was scored as follows: 0 (No loss), 1 (< 0.75 LPF), 2 (0.75 - 1.5 LPF), 3 (1.5 - 2.25 LPF), 4 (> 2.25 LPF). Epidermal cytoplasmic vacuole formation was scored 0 to 3 on the basis of size of vacuoles and extent of occurrence as follows: 0

(no vacuoles seen), 1 (small vacuoles occupying less than one-half apparent cytoplasmic volume, and 3 (vacuoles large enough to compress or displace nucleus). Subepidermal cleft formation was scored on the basis of extent of occurrence across the diameter of a low power (4X) field. 0 (none), 1 (< 25%), 2 (25% - 50%), 3 (50% - 75%), and 4 (75%-100%). Inflammation was scored from 0 to 4 as follows: 0 (none), 1 (apparent increase in PMN's in vessels and migration of these cells into the adjacent tissue in the vicinity of the vessels, 2 (more diffuse, but still relatively sparse inflammation), 3 (intermediate between 2 and 4) and 4 (maximum density of PMN's. The walling-off pattern may or may not be present.)

In a subsequent experiment, we studied the histologic changes resulting from application of four additional lewisite analogs to nude mouse skin. PDA, phenylarsine oxide, phenyldiiodoarsine, lewisite oxide and lewisite iodide were applied to the skin of nude mice (four animals per group). All arsenicals were applied in single one microliter doses as described above for PDA, except phenyldiiodoarsine. Because of its lower solubility in ethanol, five applications onto the same circular area were necessary to accomplish a total arsenical dose equivalent to the others. The animals were killed after 24 hours and the skin samples prepared as described above.

RESULTS

Macroscopic changes.

Application of PDA to human skin grafts caused marked edema within 6 hr and, after 12 hr, the edematous areas (approximately 0.7 cm in diameter) were greyish. PDA treatment of nude mouse skin caused macular erythema and slight edema within 30 min. By 3 hr the erythema disappeared but the edema was more severe. A grey-white zone appeared around the lesion within 5 hr. By 16 hr, the edema had subsided, but the grey-white zone remained.

Histologic observations: Untreated nude mouse skin and human skin grafts.

The normal untreated nude mouse epidermis (Figure 1) consisted of three or four cell layers with a thin stratum corneum. Hair follicles and sebaceous glands were present. The hair developed in apparently normal follicular structures but the hair shaft fractured near the epidermis in what appeared to be a contorted infundibulum. The dermis was not differentiated into papillary and reticular, as occurs in human skin. The normal mouse dermis was devoid of inflammatory cells. Mast cells were readily found. The panniculus carnosus (Figure 2), a thin layer of striated muscle, was separated from the dermis by a thin

layer of adipose tissue. In the usual specimen, fat was present inferior to the panniculus.

The grafted human skin departed considerably from normal (Figures 2 and 3). The epidermis exhibited the usual stratification, but rete ridges were blunted or absent. Focal ulcerations of unknown cause were occasionally seen at the graft margin. The ulcerations were histologically distinct from the lesion caused by PDA. Appendages were sparse and limited to an occasional vellus follicle and sebaceous gland. Eccrine glands were occasionally seen. The dermis had lost its usual pattern and consisted of an undifferentiated fibroblastic proliferation with an increase in connective tissue fibers. Foreign body granulomas were common, probably resulting from introduction of foreign particles during handling and surgery. There were occasional lymphoid cells.

Histologic changes following application of PDA.

Several consistent changes followed the application of PDA to nude mouse skin and human skin grafts. These are listed below in the approximate temporal order of their appearance. The changes were similar in mouse and human skin grafts except for timing of appearance and vacuole formation, the latter appearing only infrequently in the mouse skin.

* Epithelial cell nuclear changes (Figures 4 and 5) were characterized by early pyknosis (shrinkage of the nucleus) followed by loss of stainability as the cells became necrotic. The nuclear and cytoplasmic changes appeared almost simultaneously.

* Cytoplasmic changes, indicated by loss of the normal amphophilic staining quality, resulted in increased eosinophilia. Cytoplasmic vacuolization occurred in the epidermal cells. Vacuoles were larger and more numerous in the grafts. Vacuolization was not coextensive with other epidermal cytoplasmic and nuclear changes. Instead, the vacuoles appeared near the periphery of the affected area.

* Clefts (Figures 4 to 6) appeared frequently within the basement membrane zone. Fluid build-up was not apparent.

* Hair follicles and sebaceous glands showed at approximately the same time similar cellular changes as the epidermal cells. As in the epidermis, the affected cells were the more metabolically active cells.

* Dermal inflammation, consisting exclusively of PMNs, appeared after a distinct delay of 4 to 6 hr. Later, the PMNs formed a peculiar "wall" around the reaction zone but did not infiltrate the area. The "wall" was characterized by a dense band of PMNs (Figures 7 and 8) extending from the borders of the affected epidermis, through the dermis, to the underlying fat. It enclosed the altered dermis and fat in a manner similar to that seen in ischemic infarcts, although in the case of the PDA lesions, the enclosed tissue did not appear necrotic.

* Adipose tissue showed areolar degeneration (Figure 9) and was devoid of recognizable adipocytes.

* Muscle fibers in the panniculus carnosus (Figure 9) were shrunken, eosinophilic and, in some areas, decreased in number.

Figure 10 summarizes the earliest time that each structural alteration was detected. Nuclear and cytoplasmic changes occurred rapidly and at approximately the same time. Appendageal changes did not lag behind, thus indicating rapid penetration of the arsenical into skin appendages. The changes occurred somewhat later in the grafts than in the mouse skin. Inflammatory cell infiltration occurred at about 4 hr and PMN "walling off" first appeared at about 12 hr. Changes in the (mouse) adipose tissue were observed at about 6 hr. Although transient edema was detected grossly, it could not be evaluated microscopically.

To investigate the degree of toxic response as a function of time, the mice were killed and the treated areas excised 6, 12, 24 or 48 hr after application of PDA. Figures 11 to 17 summarize the progress of the histological alterations from 6 to 48 hr after PDA application. Epidermal nuclei in grafts and mouse skin progressively degenerated through 48 hr after PDA application (Figure 11). Changes in cytoplasmic staining quality were maximal within 6 hr in PDA-treated mouse skin and 12 to 24 hr in human graft (Figure 12). Epidermal cytoplasmic vacuole formation was characteristic of PDA-treated human skin grafts, but was infrequent in mouse skin (Figure 13). Vacuoles reached maximal size within 12 to 24 hr. Subepidermal cleft formation occurred as a result of PDA treatment and was maximal within 12 hr in mouse skin and 24 hr in human skin grafts (Figure 14). Nuclear degeneration in mouse hair follicles and sebaceous glands increased in severity through 48 hr (Figures 15 and 16). Human skin grafts did not contain enough appendages to evaluate quantitatively. PMN density increased through 48 hr in both nude mouse and human skin grafts (Figure 17). The PMN's did not infiltrate the lesion, but instead accumulated at the periphery of the treatment zone.

Subsequent experiments were designed to evaluate the progress of alterations after shorter exposures to PDA. Exposures of 2 and 4 hr produced obvious histologic changes, but sections obtained after only 1 hr frequently could not be distinguished from controls.

Histologic changes following application of other lewisite analogs.

After documenting the PDA-caused histologic changes in nude mouse skin, we next sought to learn whether or not the lesions of other lewisite analogs were similar. The following trivalent organic arsenicals were applied in ethanol carriers to nude mouse skin: phenylarsine oxide, phenyldiiodoarsine, lewisite oxide, lewisite iodide and PDA. At 24 hr (the only time point examined), the lesions were histologically indistinguishable from each other.

Transmission Electron Microscopic changes.

Transmission electron microscopy was used to investigate ultrastructural changes in nude mouse skin after PDA exposures of 1, 2, 4, and 6 hr. PDA caused a widening of intercellular spaces (Figure 18), although the desmosomes remained attached. Nuclear degeneration was apparent (Figure 19). Areas containing clefts clearly showed separation in the lamina lucida, with the lamina densa forming the base of the cleft (Figures 19 and 20).

DISCUSSION

Nuclear degeneration and loss of cytoplasmic basophilia in epidermal and appendageal cells were the earliest characteristic structural alterations resulting from PDA exposure. Epidermal clefts frequently occurred in both skin types, while cytoplasmic vacuoles formed primarily in human skin xenografts. PMNs appeared after 4 to 6 hr. The lesions caused by phenylarsine oxide, phenyldiiodoarsine, lewisite oxide and lewisite iodide were histologically indistinguishable from that caused by exposure to PDA. This suggests that all of the lewisite analogs examined have similar potency and biologic action.

In general, our findings support and extend the work of Cameron, Carleton and Short (6). They studied biopsies of human skin following exposure to drops of pure lewisite. Presumably this represents a dose several times higher than the PDA dose used in the present study. They described blisters in the basement membrane, eosinophilic staining of the epidermal cell cytoplasm, hydropic cytoplasm and nuclear degeneration. Appendages were not described. They apparently observed "walling off" of the PMNs: "Lying just below the floor of the blister there is often a narrow band of scattered degenerate polymorphs....its restricted nature suggests that the first leucocyte

response ceases, possibly as a result of circulatory stasis or because of unfavorable conditions" (6). Their report did not include photomicrographs.

In an earlier (1926) U. S. Army study, histologic changes were studied in the skin of horses after application of drops of lewisite (7). At 5 hr no degenerative changes were apparent in the epidermis, but some of the epithelial cells in the hair follicles contained vacuoles. (This is at variance from our results in which epidermal changes occurred early.) Edema was a prominent finding with some migration of PMNs out of blood vessels. By 24 hr, there was increased affinity for eosin and shrinkage of nuclei. Vacuolar degeneration of nuclei of individual cells occurred at the margin of the lesion. Appendages showed the same changes as the epidermis. There was PMN infiltration in all layers of dermis and subcutaneous tissue. At 48 hr, the epidermal changes had progressed to necrosis. Dermal fibroblast nuclei were pyknotic and, in the dermis; "Leucocytic infiltration is present only at the margin; over the summit of the lesion, the corium is practically free of wandering cells. The deeper layers of the corium, on the other hand, are diffusely invaded with leucocytes. These do not show the caryorrhexis which is so striking in the mustard-gas lesions" (7). Allowing for variations in times of appearance and absence of vesicles in the horse, lewisite produced remarkably similar changes to those caused by PDA in the skin of the human and nude mouse.

In this study the appearance of vacuoles, especially in the PDA-treated graft epidermis, is unexplained. Vacuoles were not distributed throughout the lesion, but instead appeared only at the periphery of the treated area. Possibly vacuole formation is dependent on metabolic processes which remain in minimally-injured cells, but have ceased in the central portion of the lesion. In 1941, Montgomery and Waisman (8) described similar changes in arsenical dermatoses but, in recent years, dermatopathologists have considered vacuolization to be nonspecific.

PMNs appeared after 4 to 6 hr, but seemed to be unable to migrate beyond a certain point, at least by 48 hr. Instead they formed a wall around the lesion. Similar "walling off" is seen in such instances as myocardial or renal infarct, where tissue is dead or dying and circulation has ceased. However, the walled-off areas in the present study did not appear infarcted, although nuclei of dermal fibroblasts were pyknotic. A possible explanation for piling up of PMNs is that the residual arsenical concentration in the treatment site was high enough to be cytotoxic or to inhibit their mobility. PDA is a potent inhibitor of several phagocytosis-related cellular processes in PMNs (McGown, unpublished observations).

Clefts in the basement membrane are presumably the histologic precursors to macroscopic blisters, although there were no obvious blisters in these experiments. A possible reason for the lack of blisters is that the follicular structures formed anchoring points and inhibited the formation of visible blisters. The basement membrane of the follicles did not separate, despite the fact that the follicular cells were damaged.

Nitrogen mustard exposure also causes basement membrane clefts in human skin grafts on nude mice (van Ravenswaay and Black, unpublished observations). Several blistering human diseases are characterized by cleft formation in the basement membrane. Because the blistering process involves the basement membrane, we are including in this discussion a review of present knowledge of its composition.

The basement membrane is a complex, but ordered, structure consisting of components from both the epidermis and dermis. An electron dense layer (lamina densa) forms a continuous foundation that is attached to the underlying dermis by anchoring fibrils extending in an irregular fashion into the dermis. Sometimes the "sublamina densa" is distinguished as the region which contains the anchoring fibrils, microfibrillar bundles and collagen fibers (9). The basal keratinocytes are separated from the lamina densa by the lamina lucida, an electron lucent layer without visible structure by transmission electron microscopy. The lamina lucida appears continuous with the intercellular spaces of epidermis, although the glycosaminoglycans of the epidermis and lamina lucida can be distinguished. Basal cells are attached to one another by means of desmosomes. In addition, hemidesmosomes in their membranes abut the lamina lucida, and from these extend delicate filaments which traverse the lamina lucida. Much investigation is currently directed toward identification of basal lamina components, primarily with immunochemical techniques. The lamina lucida contains laminin and bullous pemphigoid antigen, both of which are synthesized by the epidermis (10, 11). Laminin, a heparan sulfate-rich proteoglycan organized into disulfide-linked chains, appears to promote attachment of epidermal cells to type IV collagen (12). The bullous pemphigoid antigen is a disease-specific, disulfide-containing glycoprotein identified by antibodies circulating in patients' sera (13). The sublamina densa region contains several antigens, including the epidermolysis bullosa acquisita antigen(s) (9).

Bullous pemphigoid is an IgG-mediated autoimmune disease in which an antibody develops against the pemphigoid antigen in the lamina lucida and, by unknown mechanisms, initiates bullae formation (14). Epidermolysis bullosa letalis (Herlitz) is an hereditary disease in which defective hemidesmosomes are possibly responsible for cleft formation in the lamina lucida (15). Several other diseases in the

epidermolysis bullosa group produce blisters by defects in the basal cells, or in the anchoring fibrils of the upper dermis.

In the present study, the clefts that formed after exposure to PDA were remarkably similar to those in the published photomicrographs of bullous pemphigoid lesions (14). Perhaps an unidentified arsenical-sensitive component in the lamina lucida is responsible for the cleft phenomenon. Or possibly the arsenical damages the anchoring filaments or inhibits some critical process in the basal cell, resulting in defective production of laminin or the bullous pemphigoid antigen. Higher resolution electron microscopy might shed light on these questions.

CONCLUSIONS

Vesicating analogs of lewisite caused reproducible histological changes in both human skin xenografts and nude mouse skin. We do not know the identity of the molecular lesions or even the locations of the arsenical-sensitive sites. The nuclear and cytoplasmic changes are rather nonspecific indicators of toxic damage and disruption of homeostasis. Because of the unusual nature of the epidermal clefts, it is tempting to speculate that there is an arsenical-sensitive component in the basement membrane.

RECOMMENDATIONS

Studies should be continued to gain insight into the subcellular (and ultimately molecular) targets of arsenicals in skin. Such information is needed to assist in the design of improved antidotes.

REFERENCES

1. Gates M, Williams JW, Zapp JA. Arsenicals. In: Chemical warfare agents and related chemical problems. Summary Technical Report of Division 9, National Defense Research Committee, Office of Scientific Research and Development, 1946; vol 1.

2. Prentiss AM. Chemicals in war. New York: McGraw-Hill Book Company, 1937;177-200.

3. Webb JL. Arsenicals. In: Enzyme and metabolic inhibitors. New York: Academic Press, 1966; vol 3.

4. Reed ND, Manning DD. Long-term maintenance of normal human skin on congenitally athymic (nude) mice. Proc Soc Exp Biol Med 1973;143:350-353.

5. Krueger GG, Manning DD, Malouf J, Ogden B. Long-term maintenance of psoriatic human skin on congenitally athymic (nude) mice. J Invest Dermatol 1975;64:307-312.

6. Cameron GR, Carleton HM, Short RHD. Pathological changes induced by lewisite and allied compounds. J Pathol Bacteriol 1946;58:411-422.

7. Pappenheimer AM. Special Report No. 38. The cutaneous lesions caused by dichlorethylsulphide and lewisite in the horse. In: Medical aspects of gas warfare. Medical Department of the US Army in the World War; vol 14, US Government Printing Office, 1926;755-757.

8. Montgomery H, Waisman M. Epithelioma attributable to arsenic. J Invest Dermatol 1941;4:365-483.

9. Katz, SI. The epidermal basement membrane zone - structure, ontogeny and role in disease. J Am Acad Dermatol 1984;11:1025-1037.

10. Pruneriaa M, Regnier M, Fougere S, Woodley D. Keratinocytes synthesize basal-lamina proteins in culture. J Invest Dermatol 1983;81 (suppl) 74s-81s.

11. Briggaman RA. Biochemical composition of the epidermal-dermal junction and other basement membrane. J Invest Dermatol 1982;78:1-6.

12. Stanley JR, Hawley-Nelson P, Yarr M, Martin GR, Katz SI. Laminin and bullous pemphigoid antigen are distinct basement membrane

proteins syathesized by epidermal cells. J Invest Dermatol 1982;78:456-459.

13. Stanley JR. Identification and partial characterization of pemphigoid antigen extracted from normal skin. J Invest Dermatol 1984;82:108-111.

14. Maito K, Morioka S, Ogawa H. The pathogenic mechanisms of blister formation in bullous pemphigoid. J Invest Dermatol 1982;79:303-306.

15. Anton-Lamprecht I. Genetically induced abnormalities of epidermal differentiation and ultrastructure in ichthyoses and epidermolyses: pathogenesis, heterogeneity, fetal manifestation, and prenatal diagnosis. J Invest Dermatol 1983;81 (suppl): 149s-156s.

LIST OF FIGURES

Figure 15 Nuclear degeneration of nuclei in outer sheath and bulb of mouse hair follicles resulting from PDA exposure.

Figure 16 Degeneration of undifferentiated cells in mouse sebaceous gland resulting from PDA exposure.

Figure 17 Inflammation resulting from PDA exposure.

Figure 18 Transmission photoelectron micrograph of PDA-treated (1 hr) nude mouse epidermis illustrating widening of intercellular spaces.

Figure 19 Transmission photoelectron micrograph of PDA-treated nude mouse skin illustrating two degenerate nuclei (N) and a cleft (Cl) within the basal lamina.

Figure 20 Transmission electron photomicrograph of PDA-treated (4 hrs) nude mouse skin illustrating clefts within the basal lamina.

Figure 1. Photomicrograph of normal nude mouse skin. E = Epidermis; H = Hair follicle showing contorted infundibulum (follicular opening); S = Sebaceous gland; D = Dermis; F = Subcutaneous fat; X131.

Figure 2. Photomicrograph of normal human skin xenograft on nude mouse skin. White bar marks junction of mouse skin (left) and human skin graft (right). P = Panniculus carnosus; X33.

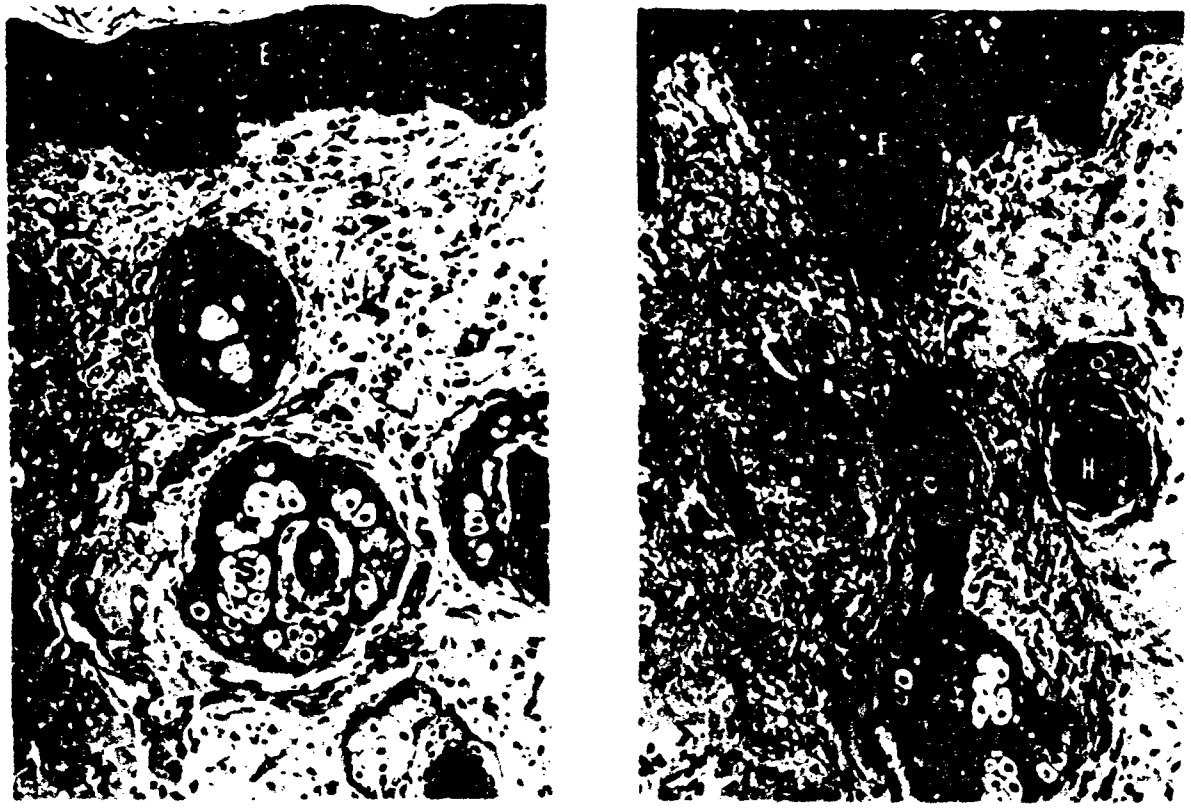

Figure 3. Photomicrographs of normal human skin xenografts illustrating hair follicles and sebaceous glands. D = Dermis; E = Epidermis; H = Vellus hair follicle (cross section of hair shaft above "H"); X 31

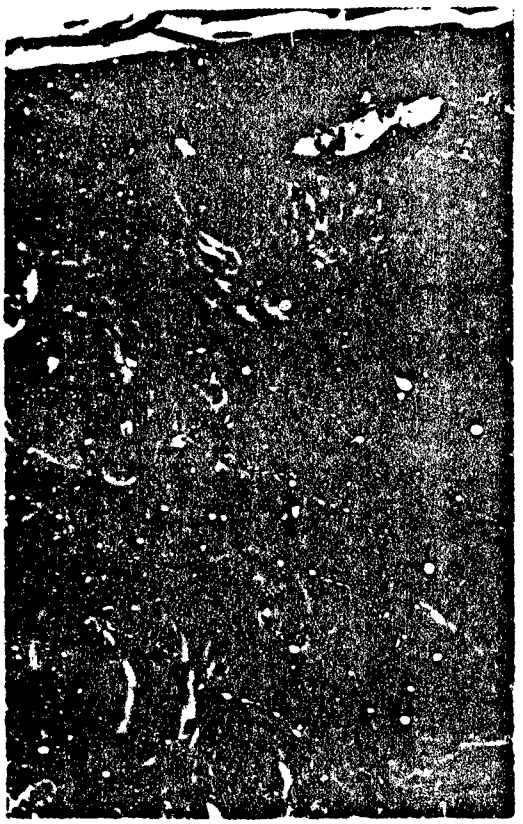

Figure 4. Photomicrograph of PDA-treated (24 hrs) human skin xenograft revealing cleft formation in the basement membrane zone. Cl = Cleft. X151.

Figure 5. Photomicrograph of PDA-treated (24 hrs) human skin xenograft revealing a cleft in the basement zone and cytoplasmic vacuoles. V = Vacuole; ↑↑↑ indicate border of affected zone and normal epidermis to right. Note sharp demarcation of affected and normal epidermis. Note also pyknotic (shrunken) and darkened nuclei in affected area. I = Inflammatory cells in dermis; Cl = Cleft. X330.

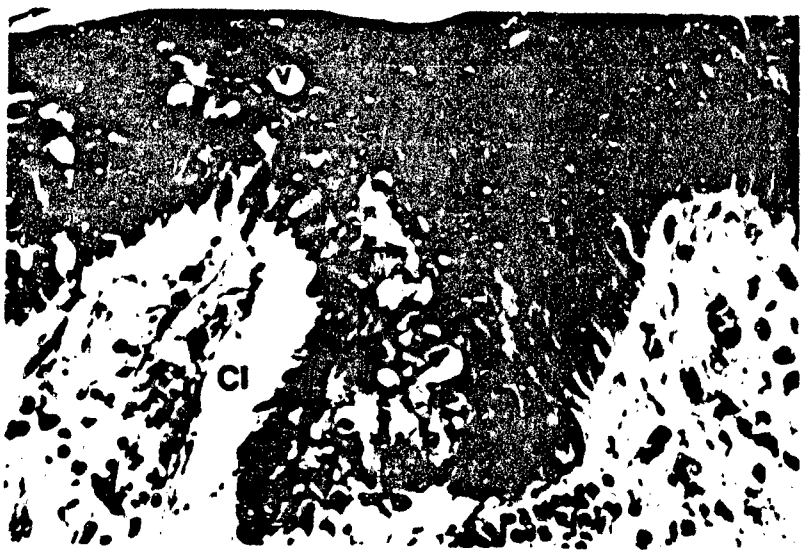

Figure 6. Photomicrograph of PDA-treated (6 hrs) nude mouse skin revealing clefts in the basement membrane zone. Cl = Cleft; H = Hair follicle; X131.

Figure 7. Photomicrograph of PDA-treated (24 hrs) human skin xenograft illustrating the "walling off" of the polymorphonuclear leukocytes around the affected skin to the left. W = Wall; X33.

Figure 8. Photomicrograph of PDA-treated (24 hrs) nude mouse skin illustrating the "wall" of polymorphonuclear leukocytes following a convoluted course. W = Wall; X33.

Figure 9. Photomicrograph of PDA-treated (24 hrs) nude mouse skin illustrating areolar changes with inflammatory reaction in the underlying muscle. D = Dermis; A = Areolar tissue; I = Inflammatory cells; M = Muscle fiber in panniculus carnosus; X131.

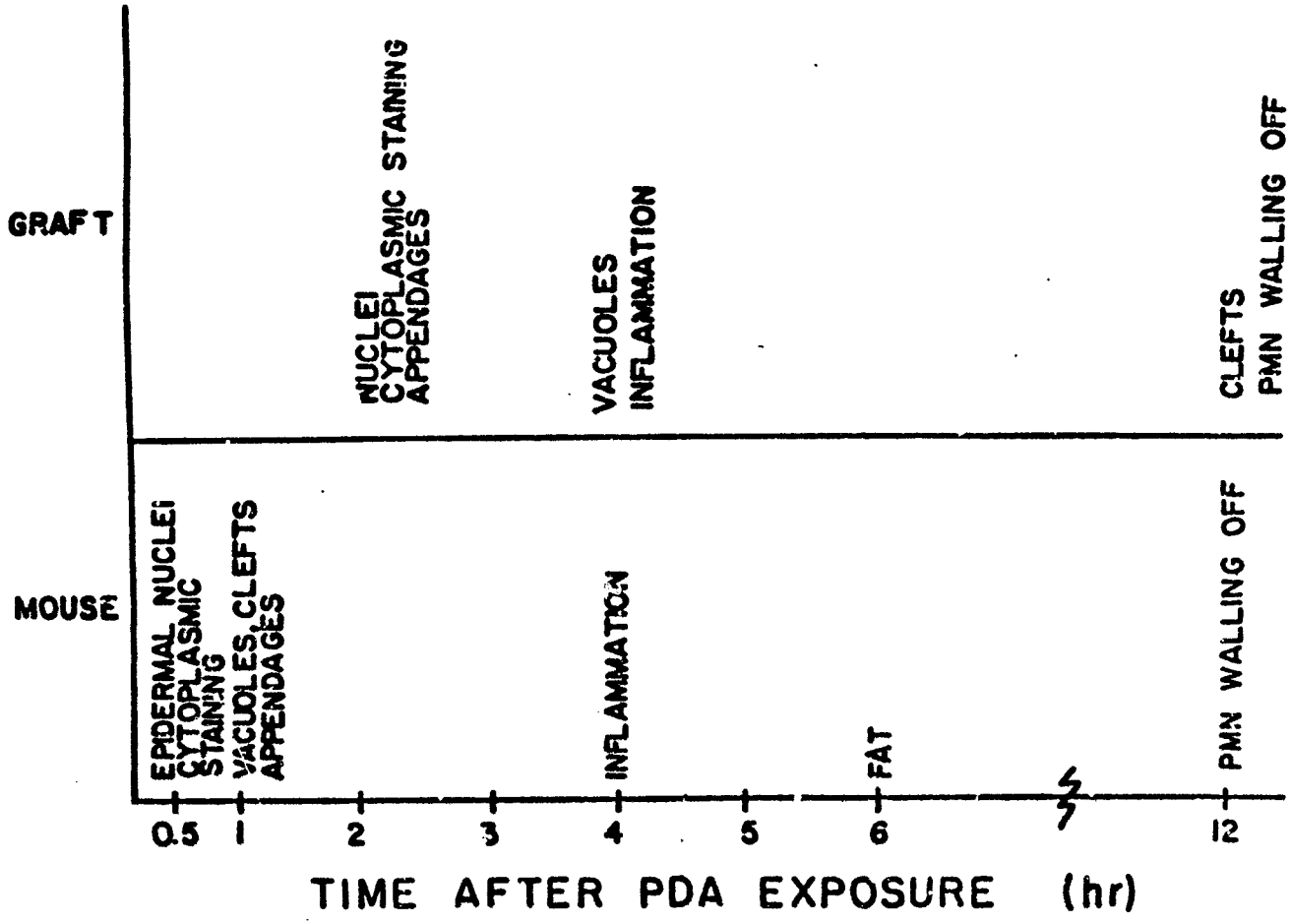

Figure 10. Time after PDA exposure at which histological cnanges were first observed in nude mouse skin and human skin xenografts.

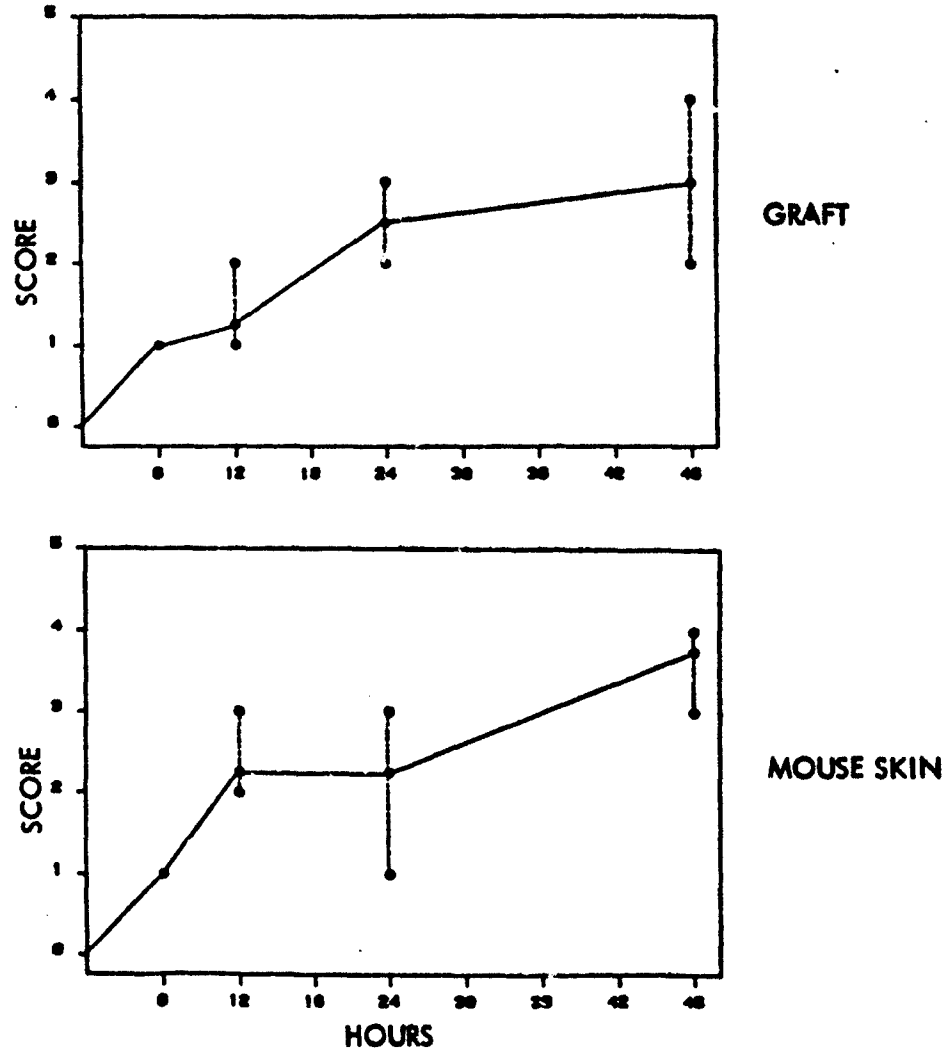

Figure 11. Epidermal nuclear degeneration after PDA application. Severity of damage was graded on a scale of 0 to 4: 0 (no change), 1,2 (mild, moderate pyknosis), 3 (loss of basophilia, with outline remaining), 4 (complete loss of nuclei. Solid circles represent mean of n = 4; vertical lines represent range when scores were not identical.

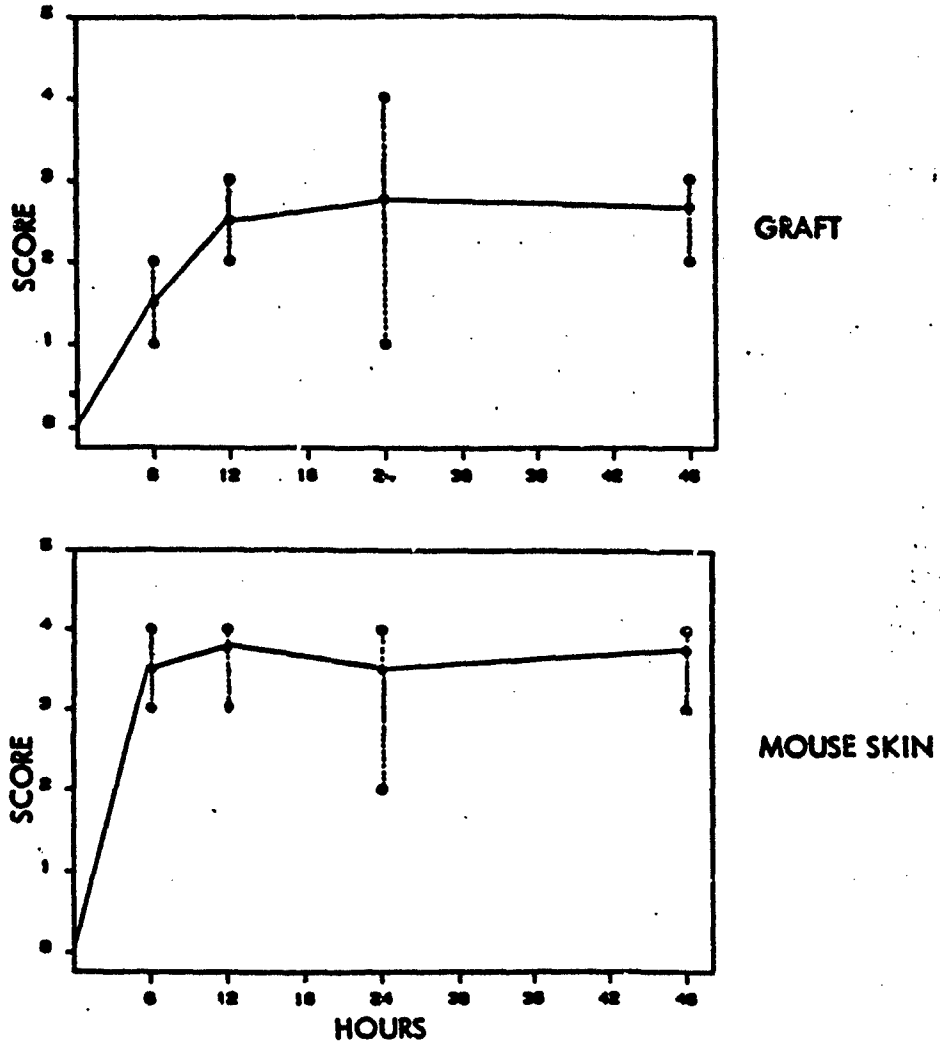

Figure 12. Loss of basophilia after PDA application. The extent of loss of cytoplasmic basophilia in 3 contiguous low power fields (LPF) (4X magnification) was scored as follows: 0 (No loss), 1 (≤ 0.75 LPF), 2 (0.75 - 1.5 LPF), 3 (1.5 - 2.25 LPF), 4 (>2.25 LPF). Solid circles represent mean of n = 4; vertical lines represent range when scores were not identical.

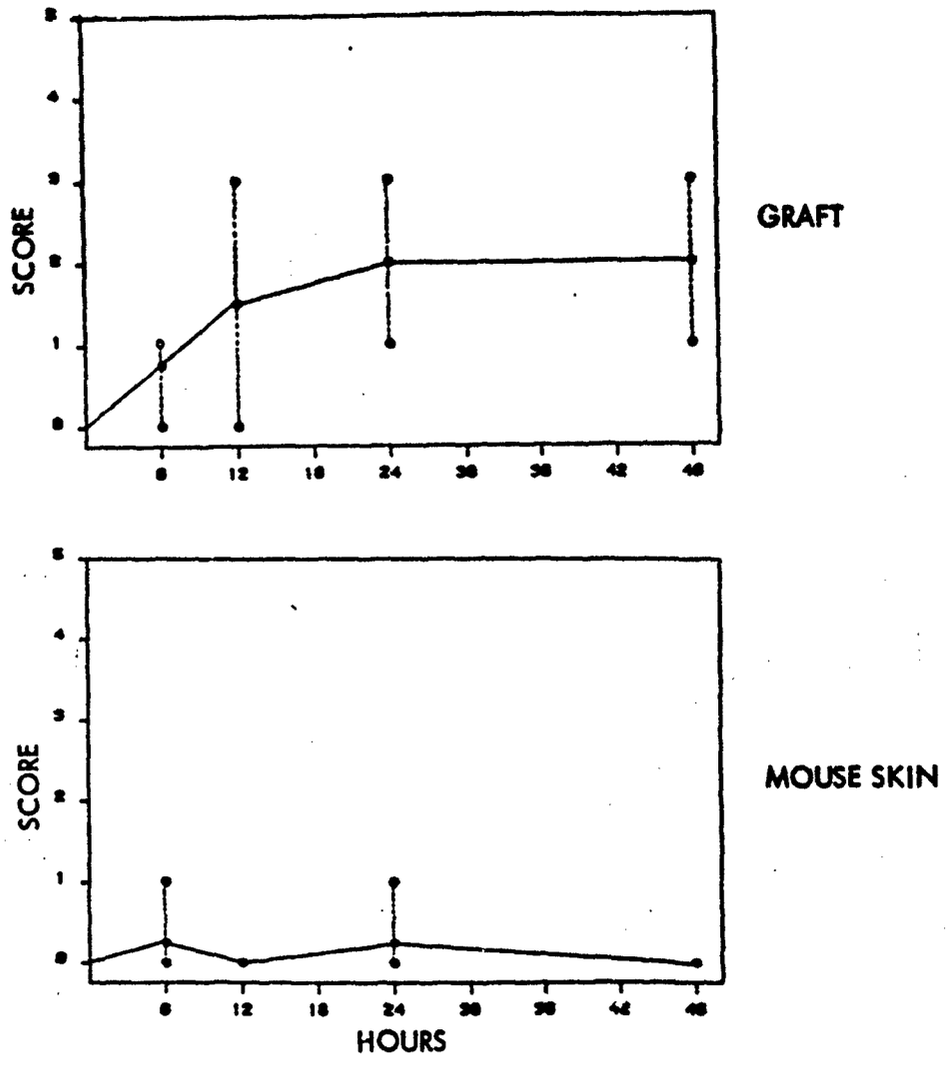

Figure 13. Epidermal cytoplasmic vacuole formation resulting from PDA application; scored 0 to 3 based on size of vacuoles: 0 (no vacuoles), 1 (small vacuoles occupying less than one-half apparent cytoplasmic volume, 2 (vacuoles occupying more than one-half apparent cytoplasmic volume and 3 (vacuoles large enough to compress or displace nucleus. Solid circles represent mean of n = 4; vertical lines represent range when scores were not identical.

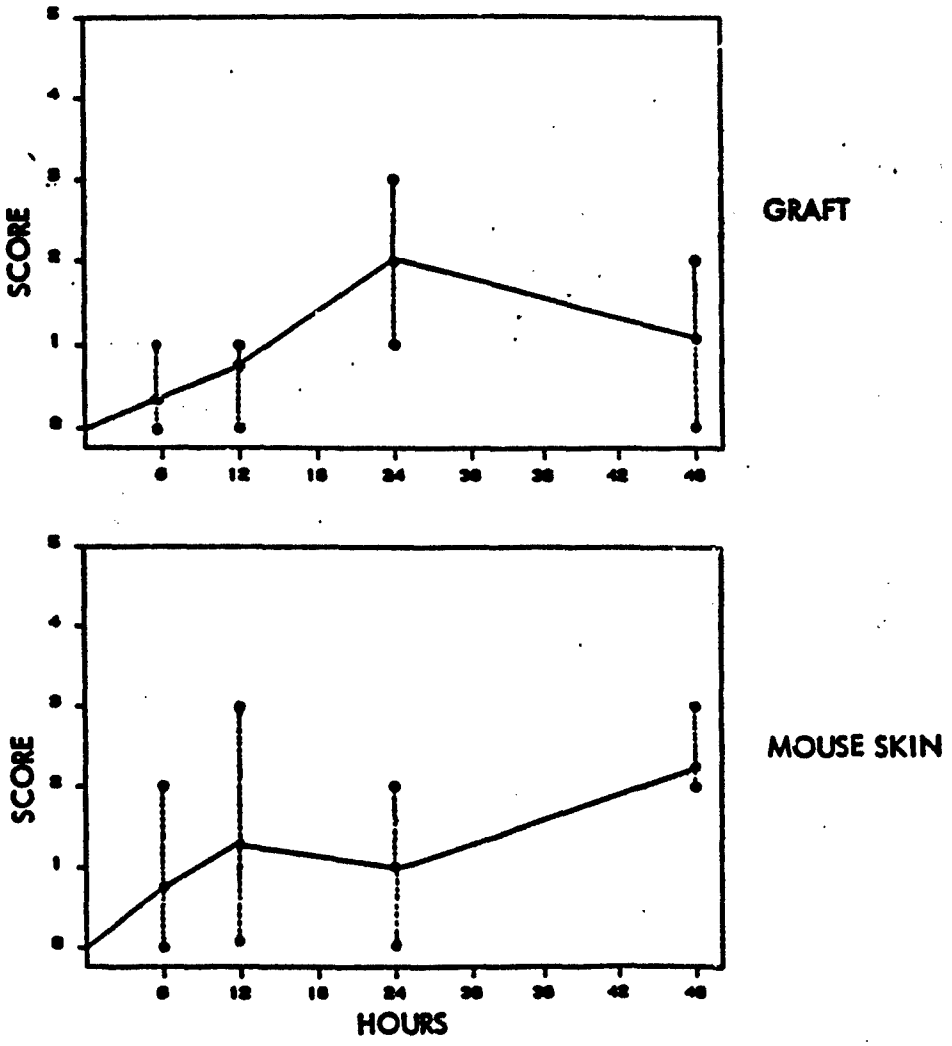

Figure 14. Subepidermal cleft formation (separation of epidermis from epidermis) due to PDA exposure. Scored on the basis of extent of occurrence across the diameter of a low power (4X) field. 0 (none), 1 (< 25%), 2 (25% - 50%), 3 (50% - 75%), and 4 (75%-100%). Solid circles represent mean of n = 4; vertical lines represent range when scores were not identical.

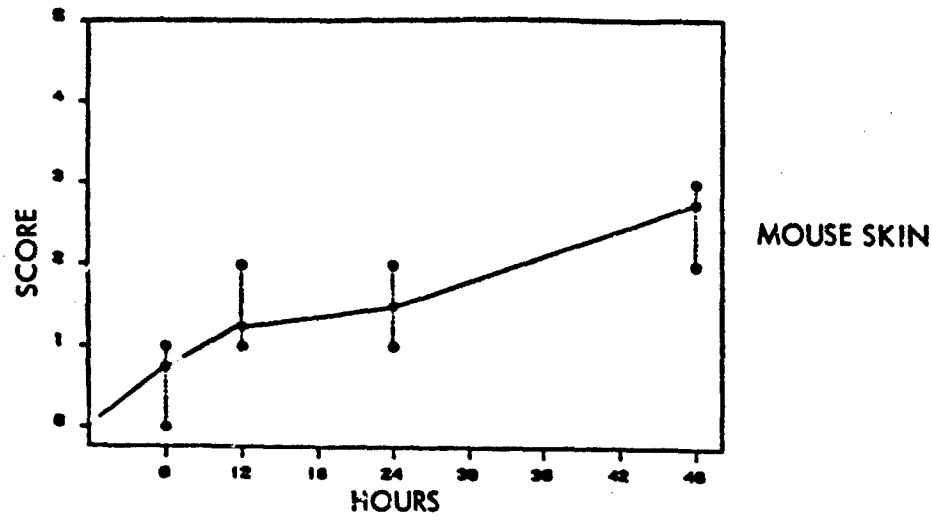

Figure 15. Nuclear degeneration of nuclei in outer sheath and bulb of mouse hair follicles resulting from PDA exposure. Scored 0 to 4 as for epidermal nuclear changes in Figure 1f. Solid circles represent mean of n = 4; vertical lines represent range when scores were not identical.

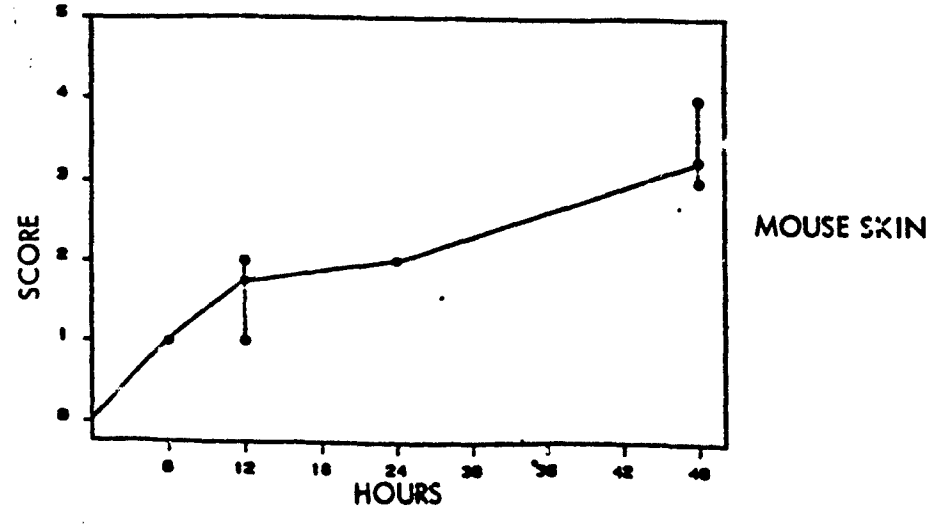

Figure 16. Degeneration of undifferentiated cells in mouse sebaceous gland resulting from PDA exposure. Changes scored 0 to 4 as for epidermal nuclear changes in Figure 1f. Solid circles represent mean of n = 4; vertical lines represent range when scores were not identical.

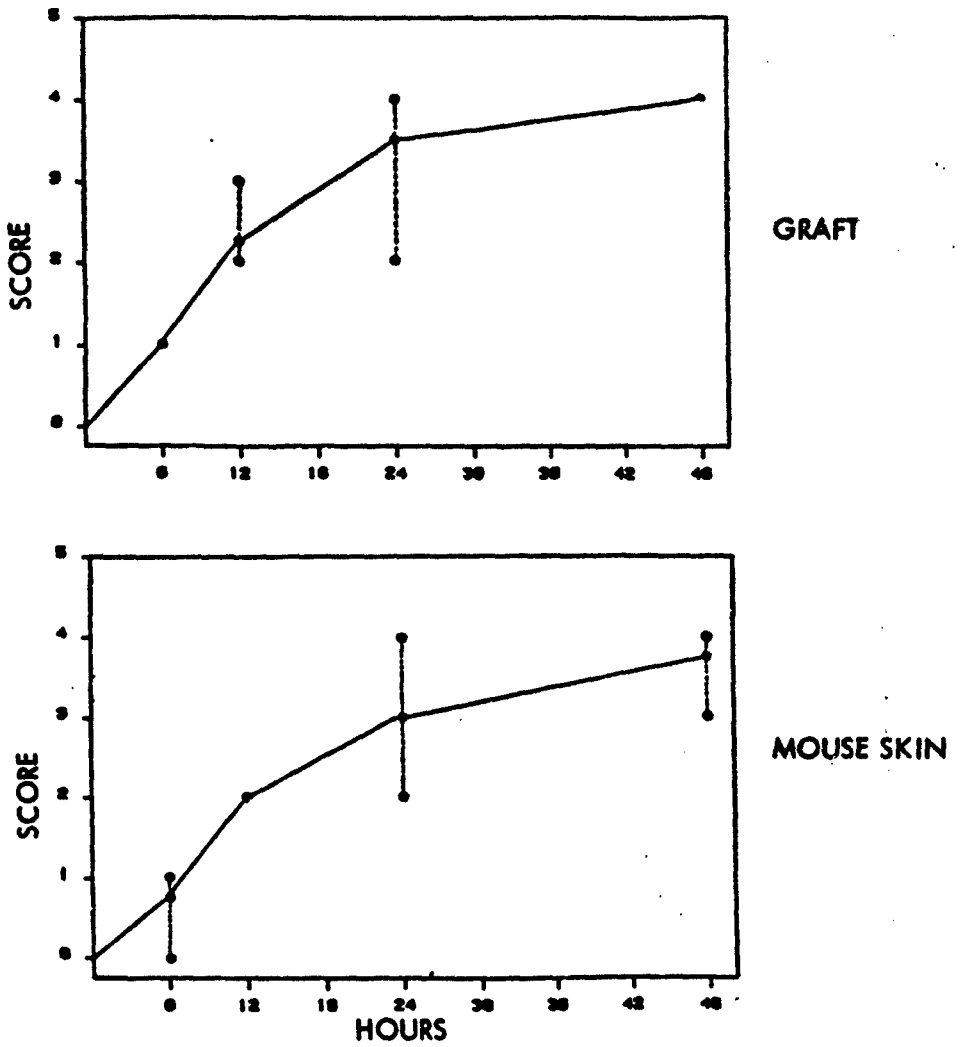

Figure 17. Inflammation resulting from PDA exposure. Scored from 0 to 4: 0 (no inflammation), 1 (apparent increase in PMN's in vessels and migration of these cells into adjacent tissue in the vicinity of the vessels), 2 (more diffuse, but still relatively sparse inflammation), 3 (intermediate between 2 and 4) and 4 (maximum density of PMN's). Solid circles represent mean of n = 4; vertical lines represent range when scores were not identical.

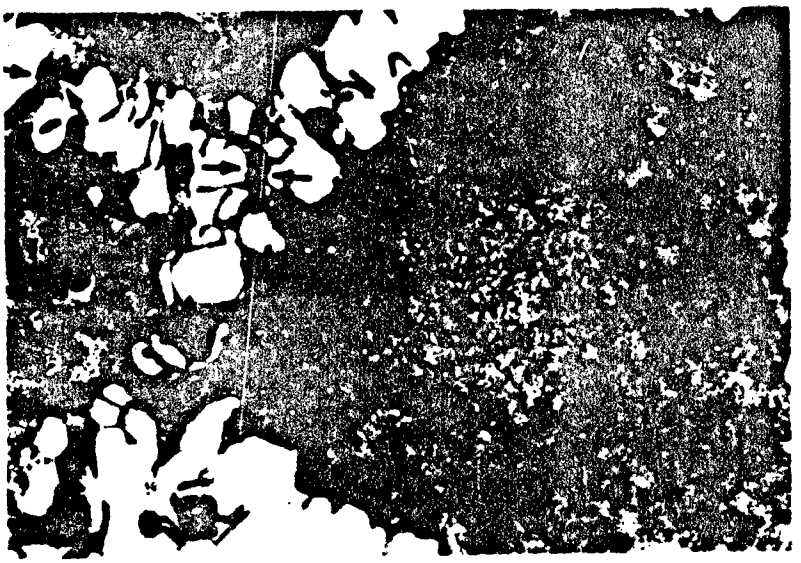

Figure 18. Transmission photoelectron micrograph of PDA-treated (1 hr) nude mouse epidermis illustrating widening of intercellular spaces. N = Keratinocyte nucleus; Cy= Keratinocyte cytoplasm; → ← = Desmosomes between arrows; X12,000.

Figure 19. Transmission photoelectron micrograph of PDA-treated nude mouse skin illustrating two degenerate nuclei (N) and a cleft (Cl) within the basal lamina. D = Dermis; X5400.

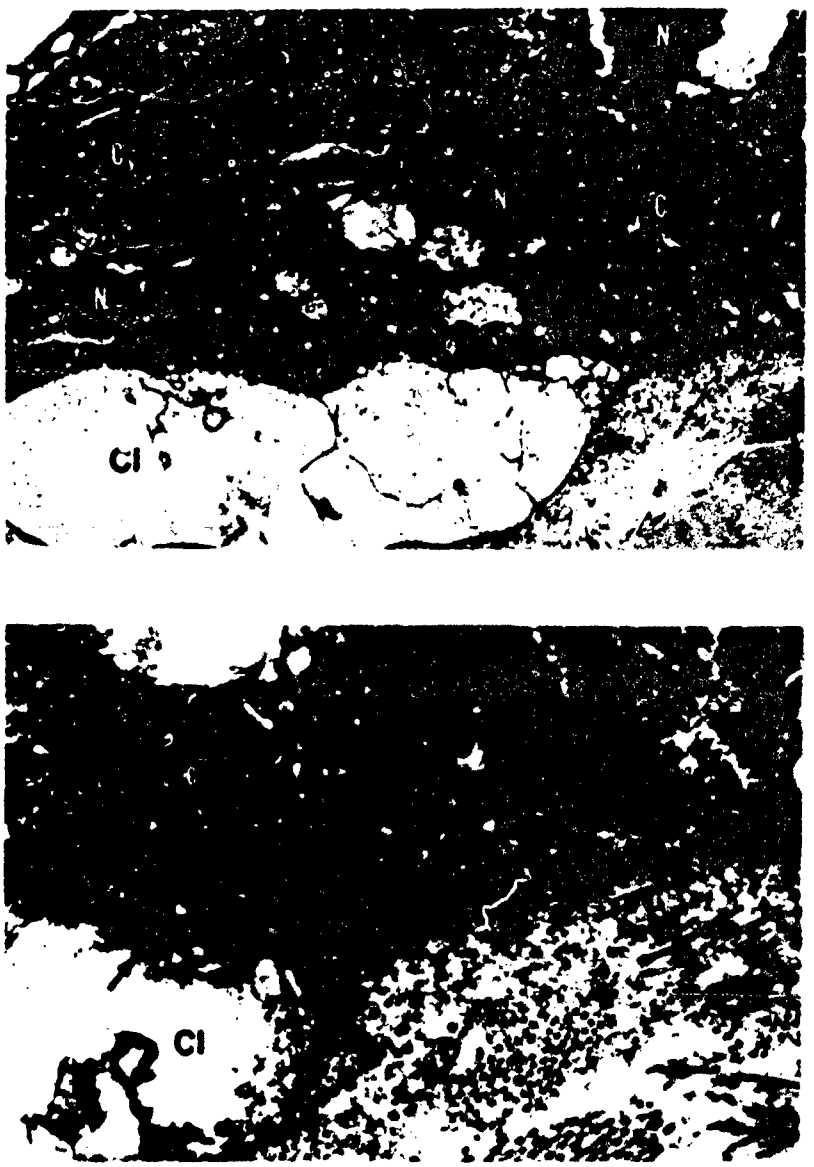

Figure 20. Transmission electron photomicrograph of PDA-treated (4 hrs) nude mouse skin illustrating clefts within the basal lamina. Cl = Cleft; D = Dermis (collagen fibers in cross and longitudinal section); K = Keratinocyte cytoplasm; Cy = Cytoplasm of keratinocyte; N = Nucleus of Keratinocyte. Double arrows (⇉) indicate lamina densa with lamina lucida just above, single arrow (↗) indicates detached hemidesmosome. Top, X5400; Bottom, X18,000.

OFFICIAL DISTRIBUTION LIST

Commander
US Army Medical Research
and Development Command
ATTN: SGRD--RMS/Mrs. Madigan
Fort Detrick, MD 21701-5012

Defense Technical Information Center
ATTN: DTIC/DDAB (2 copies)
Cameron Station
Alexandria, VA 22304-6145

Office of Under Secretary of Defense
Research and Engineering
ATTN: R&AT (E&LS), Room 3[illegible]29
The Pentagon
Washington, DC 20301-3080

The Surgeon General
ATTN: DASG--TLO
Washington, DC 20310

HQ DA (DASG--ZXA)
WASH DC 20310-2300

Commandant
Academy of Health Sciences
US Army
ATTN: HSHA--CDM
Fort Sam Houston, TX 78234-6100

Uniformed Services University
of Health Sciences
Office of Grants Management
4301 Jones Bridge Road
Bethesda, MD 20814-4799

US Army Research Office
ATTN: Chemical and Biological
Sciences Division
PO Box 12211
Research Triangle Park, NC 27709-2211

Director
ATTN: SGRD--UWZ--L
Walter Reed Army Institute
of Research
Washington, DC 20307-5100

Commander
US Army Medical Research Institute
of Infectious Diseases
ATTN: SGRD--ULZ--A
Fort Detrick, MD 21701-5011

Commander
US Army Medical Bioengineering
Research & Development Laboratory
ATTN: SGRD--UBG--M
Fort Detrick, Bldg 568
Frederick, MD 21701-5010

Commander
US Army Medical Bioengineering
Research & Development Laboratory
ATTN: Library
Fort Detrick, Bldg 568
Frederick, MD 21701-5010

Commander
US Army Research Institute
of Environmental Medicine
ATTN: SGRD--UE--RSA
Kansas Street
Natick, MA 01760-5007

Commander
US Army Institute of Surgical Research
Fort Sam Houston, TX 78234-6200

Commander
US Army Research Institute
of Chemical Defense
ATTN: SGRD--UV--AJ
Aberdeen Proving Ground, MD 21010-5425

Commander
US Army Aeromedical Research Laboratory
Fort Rucker, AL 36362-5000

AIR FORCE Office of Scientific
Research (NL)
Building 410, Room A217
Bolling Air Force Base, DC 20332-6448

Commander
USAFSAM/TSZ
Brooks Air Force Base, TX 78235-5000

Head, Biological Sciences Division
OFFICE OF NAVAL RESEARCH
800 North Quincy Street
Arlington, VA 22217-5000