Lab. Project 9400-12, Progress Report 15



# Histochemical Studies of Skin Burns, Contact and Flash Lamp Burns DASA Subtask 03.062

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# HISTOCHEMICAL STUDIES OF SKIN BURNS,

# CONTACT AND FLASH LAMP BURNS

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## ABSTRACT

Histochemical studies to evaluate the effects on enzyme activity as a possible cause of cell damage in thermal injury were made on the skin of white rats after contact with a copper block at 60°C for 5 seconds and after exposure to thermal radiation of 3.7 $cal/cm^{-2}$ from a xenon flash lamp pulse peaking in 0.5 milliseconds. The hot copper contact burn caused deep burn lesions with vascular thrombosis. There was no loss of enzyme activity until 4 hours after the burn exposure. The flash lamp exposure showed only shallow tissue damage after 24 hours. There was immediate loss of enzyme activity in the surface epithelium with rapid cell death.

## SUMMARY

Since the cellular and tissue sites of critical damage in burn lesions are not known, this study was made to evaluate the possibility that enzymatic activity is destroyed.

Frozen tissue sections excised from second degree burn lesions on the depilated dorsal skin of rats were studied by standard histochemical techniques for succinic dehydrogenase (SD), lactic dehydrogenase (LD), and diphosphopyridine nucleotide diaphorase (DPND).

Sections were also stained with hematoxylin and eosin (H&E) for routine evaluation of pathological changes.

It was observed that, for copper block burns, the loss of enzymatic action was not an early finding in irreparably damaged tissue but became evident between 4 and 24 hours in conjunction with other evidences of tissue damage. This agrees with results reported by other authors that anoxic damage does not lead to early loss of enzyme activity.

The results with 3.7 cal/cm$^2$, 0.5 millisecond bursts of radiant energy were strikingly different. Biopsy specimens, examined immediately after burn, showed loss of the two dehydrogenases and the DPN diaphorase from the surface epithelium. Dermal injury was not evident and the epithelium regenerated by the third day when it showed return of enzymatic activity. The epithelium was rapidly killed but the cells were not disrupted.

Future work will continue the study of biological reactions of cellular and sub-cellular structures and functions of the skin to bursts of radiant energy.

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FIGURES

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FIGURES

## ADMINISTRATIVE INFORMATION

This work was conducted as part of the Naval Applied Science Laboratory's studies of the mechanism of thermal injury sponsored by the Defense Atomic Support Agency under DASA Subtask NWER 03.062. The casualty prediction model, being developed by NASL, includes studies of the physical mechanisms of thermal injury and requires input from studies such as those presented herein. The site and nature of thermal skin injury are inadequately known. Correlation of injury with causative temperatures requires that studies of basic mechanisms be made under controlled experimental conditions such as prevail at NASL. Normally, studies of the type reported herein which are biological in nature would not be conducted at the Naval Applied Science Laboratory. The specific problem is an important adjunct to the results of physics-oriented studies. Dr. Mixter (also a DASA consultant) conceived the study and furnished the necessary expertise in the histochemical aspects of the problem.

## ACKNOWLEDGMENTS

The work was conducted under the direction of W. L. Derksen, Senior Task Leader, and T. I. Monahan, Head, Physics Branch. George Mixter, Jr., M.D. is a consultant to this Laboratory on the medical aspects of thermal injury. Nan Pillsbury was a former member of the Laboratory staff.

## INTRODUCTION

It has long been evident that a skin burn is a complex, dynamic lesion that only begins with thermal trauma. From this point it develops, with greater or lesser effects upon every system of the body, until either the body heals the defect or death occurs.

Much progress has been made in recent years in the understanding and treatment of the systemic effects of extensive burns. Support of hemodynamic, immunologic and endocrine systems can prolong life and, although current statistics do not all support the contention, it appears likely that mortality can be lowered below the figures of a hundred or more years ago.

Little advance has been made in deriving a clearer understanding of the pathogenesis of the local lesion from which the general manifestations stem. The most notable exception to this statement is, of course, the remarkable series of studies by Henriques and Moritz[1] nearly two decades ago. This monumental attack, based on a solid foundation in the physics of heat flow, produced the concept of a "punishment integral", which is a summation of the accruing thermal damage to the cell, up to the "point of no return", beyond which the tissue cannot recover. This concept is based upon a rate process theory which has been developed by Fugitt,[2] who has given explicit expression to many of the physiochemical and thermodynamic concepts which were implicit in Henriques' approach. Since such an approach depends on extremely accurate knowledge of time-temperature histories in depth, the recent advances in

this regard by Hottel and his associates at MIT,[3,4] and particularly, by T. P. Davis[5] at the University of Rochester have been of the greatest importance.

To obtain data suitable for computer solutions of this complex time-temperature problem, a simple, homogenous population of mammalian epithelial cells was selected for a quantitative study of thermal sensitivity. Reaction rate constants were obtained[6] for death at elevated temperatures of the cells in this artificial and over-simplified system. Skin itself is a highly complex structure involving blood vessels, collagen and other elements, whose vulnerability to heat is not known. Blood vessels and lymphatics alike become thrombosed (Child and Mixter, unpublished data) very early in the development of the burn lesion. To date it is not known to what extent this factor contributes to the pathogenesis or whether it is merely a subordinate or ancillary change which indicates the extent of the lesion.

The work with tissue slices, divorced from problems of blood supply of Falls Hershey and his associates[7] suggests that there may be minor degrees of thermal trauma which can temporarily cripple the cells (as evidenced by failure to take up oxygen), but which are insufficient to produce irremediable damage and death. Such investigations point out a basic gap in our understanding of thermal trauma - the nature of the metabolic dysfunction caused by heat. At temperatures above 100°C the generation of steam physically disrupts the cell. But at lower temperatures, immediately after the "insult", standard techniques fail to show gross alterations in irretrievably damaged areas. Recently, Hinshaw has adduced additional evidence[8] of the dynamic nature of local burn pathology, which emphasizes the fact that under certain circumstances the thermal "insult" may not cause irremediable damage.

The presently reported study arose as part of a broader investigation into thermal effects upon various components of skin. The early involvement of oxidative mechanisms suggested a histochemical investigation of burned tissue - of the three enzymes involved in the Krebs cycle, and known to be in some degree sensitive to temperature elevation.

## OBJECT

The object of this investigation is to study the nature of local burn pathology. Particular emphasis is placed on the use of standard histochemical techniques to determine the temperature sensitivities of the enzymes succinic dehydrogenase (SD), lactic dehydrogenase (LD) and diphosphopyridine nucleotide diaphorase (DPND).

## METHOD

The thermal insult for the first portion of the study was provided by a hollow copper block, five mils in thickness, through which water was circulated at the desired temperature at a rate of 600 cc/min (Figure 1). The block was held in firm contact with the skin on the dorsal area of depilated Sprague-Dawley rats for various time intervals timed by a stopwatch. The rats, with a body weight of approximately 240 gms, were previously anesthetized by

intra-peritoneal administration of veterinary Nembutal (40 milligrams/kilogram of rat body weight). Table 1 shows the temperatures and times employed, as well as the punishment integral value ($\Omega$), as approximated from previous HeLa cell studies, for the surface of the skin and at a depth of 100 microns.

TABLE 1

| Temperature (°C) | Time (sec) | Punishment Integral ($\Omega$) at Surface | Punishment Integral ($\Omega$) at 100$\mu$ Depth |
|---|---|---|---|
| 60.3 | 5 | 0.6 | 0.38 |
| 60.3 | 10 | 1.3 | 0.86 |
| 58.4 | 15 | 0 9 | 0.68 |
| 58.4 | 30 | 2.0 | 1.60 |

Animals were sacrificed at 4, 24, 48 and 72 hours after the burn. The skin areas were excised, identified and dropped in liquid nitrogen. Specimens were kept bottled in a cryostat at -20°C until ready for cutting and processing. In a more recent set of observations, high-speed flash-tube exposures of rat skin were also made. The tube is a xenon-filled helix (G.E. FT-623) through which a single electrical impulse is discharged. The impulse is obtained from a parallel bank of 26 condensers of 100 mfd each, charged to 3200 volts. This discharge of approximately 12,000 joules delivers a light impulse peaking in 0.5 millisec, and decaying to 20 percent of peak irradiance in 4 millisec (Figure 2).

Rats were positioned at distances from the source which calorimetric calibration had determined to yield 3.7 and 4.6 cal/cm$^2$ total radiant exposures. Such exposures yielded extremely shallow second degree burns which became apparent only after 24 hours and healed between four and seven days.

The four "standard" copper block burns were also placed on these rats and biopsies were removed and studied according to the previously described schedule. Tissues were sectioned at 7-20$\mu$ and stained for succinic dehydrogenase,[10] lactic dehydrogenase[11] and diphosphopyridine nucleotide diaphorase.[12] Routine hematoxylin and eosin sections were also made.[9] Portions of each tissue sample were retained for further study by other techniques. Photomicrographs were taken from representative areas of each stained biopsy.

## RESULTS

The photomicrographs are shown in Figures 3a through 3d and 4a through 4d. In no instance after the copper block exposure was the destruction of enzyme activity observed as the earliest evidence of damage. In every burn studied there was eventual loss, to a greater or lesser extent, of enzyme activity in the irreparably damaged areas. Such losses began to occur between the 4th and 24th hour after the burn, and in most instances continued to progress through the 72nd hour. This statement is equally applicable to epithelial, fibroblastic, vascular and muscular cell elements. In this regard, it is interesting to note a parallelism with the recent observations of Nachlas and associates in experimental myocardial infarcts.[13] In these lesions, which are primarily anoxic in origin, enzymatic activity only began to decline some 5 or 6 hours after coronary ligation when other evidences of infarction were also beginning to become evident.

There are, of course, a vast number of respiratory enzymes, coenzymes and their more or less closely associated proteins, the majority of which are not currently amenable to study by histochemical methods. The negative findings of this report, concerning only three of them, can by no means be construed to deny that some other enzymes may indeed be the "locus minoris resistentiae" of the cell, so far as heat is concerned. But the evidence is that the enzymes studied are relatively sturdy, and that where heat is concerned, something else seems to be more vulnerable.

The early appearance of vascular thrombosis, together with the similarity of the time schedule with that of the lesions described by Nachlas, may be highly significant. It is also interesting that calculations of the punishment integral based upon epithelial cell sensitivity indicate that, at a given depth in tissue, irreversible damage has occurred where the calculated thermal insult was too mild to have produced immediate cell death.

Quite another set of phenomena was observed in the skin exposed to the high-speed flashes. The results in this series were somewhat startling. As previous studies had indicated, the immediate biopsies of the 58°C and 60°C burns showed no loss of enzyme from the skin, and no detectable lesion on staining with hematoxylin and eosin. By the 3rd day, however, the "classical" pathology had developed as previously noted.

The high-speed flash burns presented the reverse picture; the earliest biopsies revealed loss of both dehydrogenases and diaphorase from the surface epithelium and for short distances down the hair follicles. No evidence of a burn lesion developed in the dermal structures, and by the 3rd day, regenerating epithelium almost completely covered the dermis, in most cases beneath the necrotic and still adherent epithelium.

## DISCUSSION

For reasons cogently discussed by Davis,[14] it is not possible at the present time either to measure, or to calculate with great exactitude, the time-temperature curves at depth in very rapidly irradiated diathermanous skin. Nevertheless, it is certain that the maximum temperature attained in the epidermis in the present instance should have been on the order of 90°C rather than 60°C as in the copper block case. The brevity of the temperature elevation, and the sharp reduction of the peak temperature with increasing depth presumably account for the shallowness and rapid healing in this type of injury. The loss of enzyme activity in the surface epithelium is of great interest, indicating as it does that temperatures in the range between 60°C and 100°C are capable of inactivating this type of protein in extremely short times. This loss of activity, furthermore, is associated with very rapid death of the cell, though not with mechanical disruption, as seen in the "normal" appearance of the H&E stained tissue.

## SUMMARY OF RESULTS

The results of the investigation may be summarized as follows:

### Copper Block Burns

a. A 5-second contact with a copper block at 60°C caused deep, second-degree burn lesions in dorsal rat skin. The burns contained irreparably damaged areas.

b. The appearance of vascular thrombosis, evident in immediate biopsies, suggested a possible contribution of anoxic origin for these lesions.

c. The destruction of enzyme action and damage to epithelial, fibroblastic, vascular or muscular cell elements was not evident in immediate biopsies. However between 4 and 24 hours after the application of heat, loss of enzyme activity, and damage to the other cell elements became evident in the irreparably damaged areas.

### High-Speed Flash Burns

a. Flash exposures having total irradiances of 3.7 and 4.6 cal/cm$^2$ caused extremely shallow second degree burns in dorsal rat skin. The maximum temperature attained in the epidermis was estimated to be 90°C. Damage was apparent only after 24 hours and was healed rapidly between 4 and 7 days.

b. Immediate biopsies showed loss of enzyme activity in the surface epithelium and for short distances down the hair follicles. This loss of activity was associated with rapid cell death, though not with mechanical disruption.

c. Temperatures between 60°C and 90°C are capable of inactivating the three enzymes studied (succinic dehydrogenase, lactic dehydrogenase and diphosphopyridine nucleotide diaphorase) in extremely short times.

## FUTURE WORK

Investigations of the mechanism of thermal injury will be continued. Future efforts will be concerned with the biological reactions of cellular and subcellular structures and functions of the skin to bursts of radiant energy.

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PHOTO L-19749

Figure 1 - Copper block thermal exposure applicator with plastic tubing for circulating hot water.

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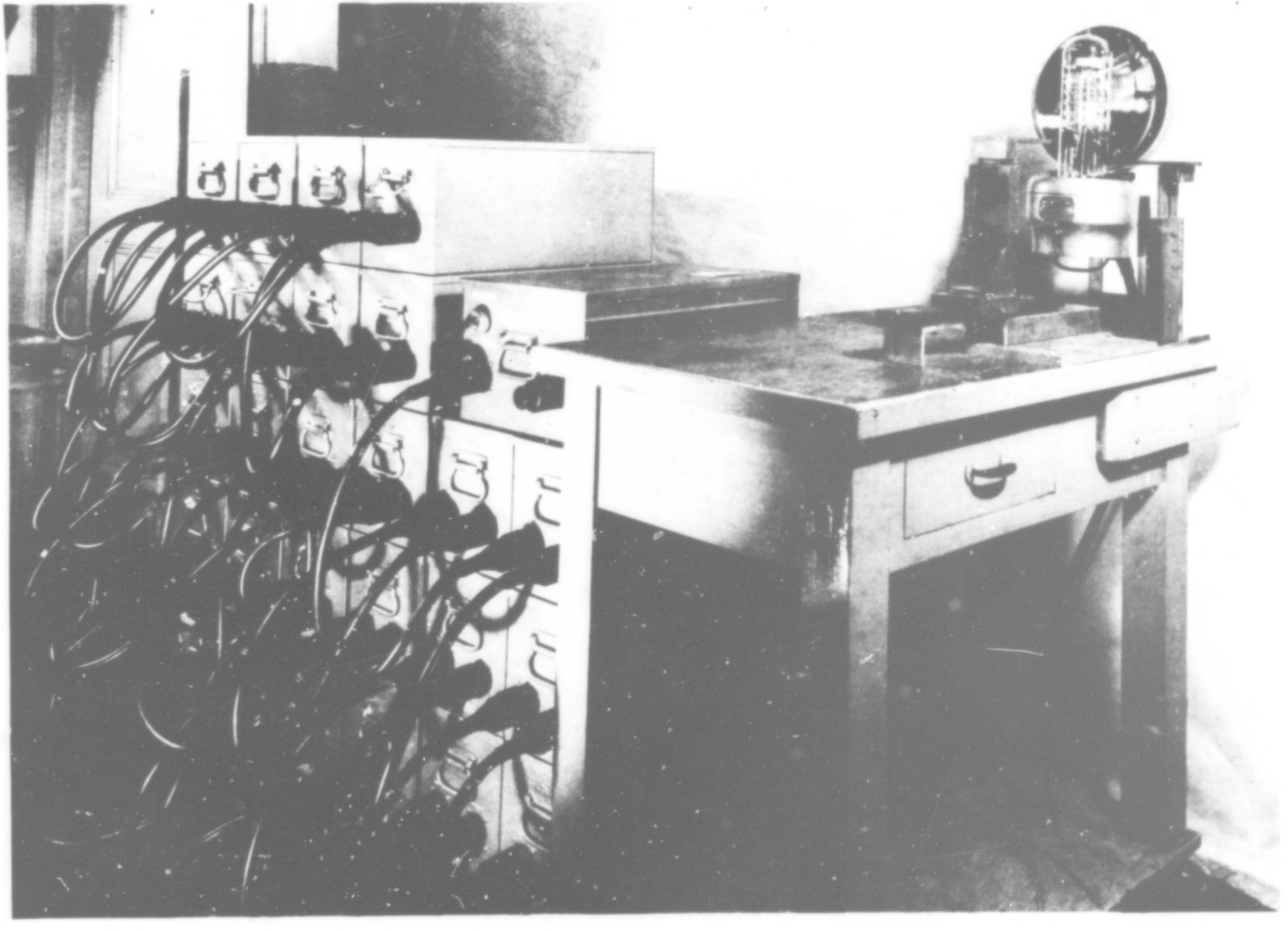

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Figure 2 - NASL Xenon flash-tube, condenser bank and associated circuitry used to produce thermal burns.

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Figure 3a. Rat skin, copper-block exposure to 60°C for 5 sec., immediate biopsy, H&E stain. Damage not evident.

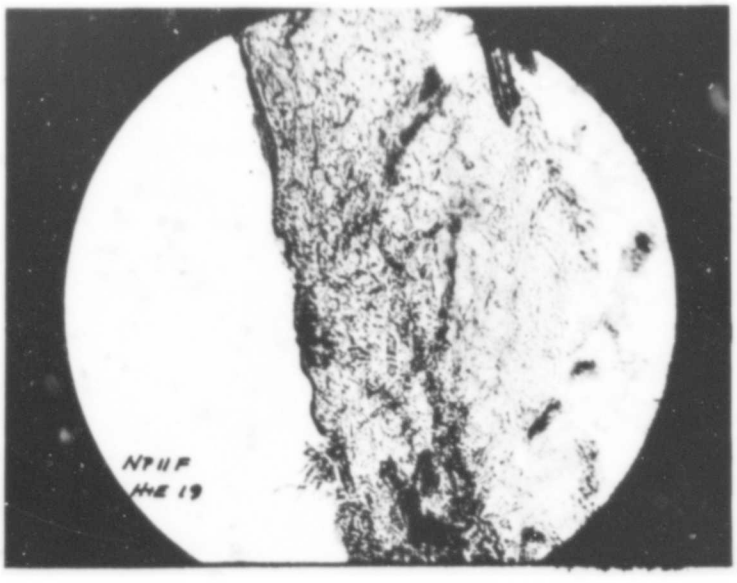

Figure 3b. Rat skin, copper-block exposure to 60°C for 5 sec., 3 day biopsy, H&E stain. Note development of deep second degree lesion.

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Figure 3a - Upper
Figure 3b - Lower

Figure 3c. Rat skin, copper-block exposure to 60°C for 5 sec., immediate biopsy, DPND stain. Tetrazolium reaction present in epithelium.


Figure 3d. Rat skin, copper-block exposure to 60°C for 5 sec., 3 day biopsy, DPND stain. Note loss of tetrazolium reaction deep into dermis.

PHOTO L-21242-2

Figure 3c - Upper
Figure 3d - Lower


Figure 4a. Rat skin, exposed to 3.7 cal/cm$^2$ flash, immediate biopsy, H&E stain. Note absence of explosive vesicles on surface.


Figure 4b. Rat skin, exposed to 3.7 cal/cm$^2$ flash, 3 day biopsy, H&E stain. Note regeneration and hyperplasia of surface epithelium.

PHOTO L-21242-3

Figure 4a - Upper
Figure 4b - Lower

Figure 4c. Rat skin, exposed to 3.7 cal/cm$^2$ flash, immediate biopsy, LD stain. Note loss of tetrazolium reaction in surface epithelium and follicles.

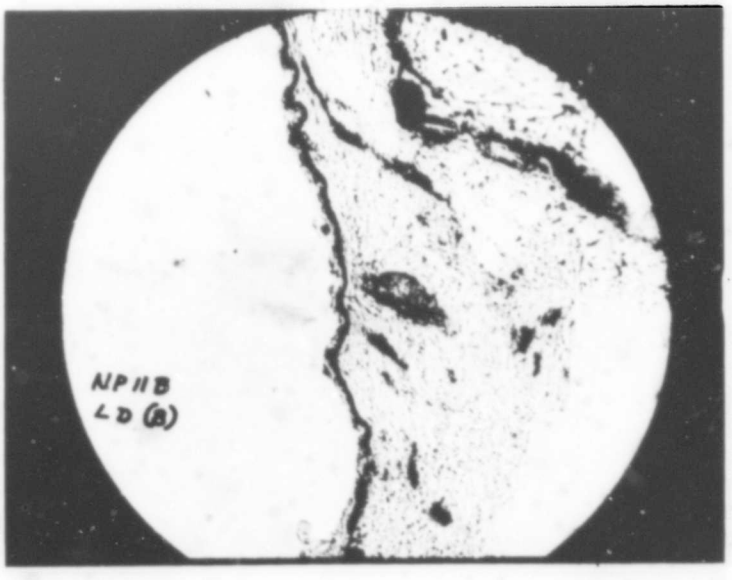


Figure 4d. Rat skin, exposed to 3.7 cal/cm$^2$ flash, 3 day biopsy, LD stain. Note return of enzyme activity in epithelial cells.

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Figure 4c - Upper
Figure 4d - Lower

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13. ABSTRACT

Histochemical studies to evaluate the effects on enzyme activity as a possible cause of cell damage in thermal injury were made on the skin of white rats after contact with a copper block at 60°C for 5 seconds and after exposure to thermal radiation of 3.7 cal/cm$^{-2}$ from a xenon flash lamp pulse peaking 0.5 milliseconds. The hot copper contact burn caused deep burn lesions with vascular thrombosis. There was no loss of enzyme activity until 4 hours after the burn exposure. The flash lamp exposure showed only shallow tissue damage after 24 hours. There was immediate loss of enzyme activity in the surface epithelium with rapid cell death.

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| 3. Cell Damage | | | | | | |
| 4. Thermal Injury | | | | | | |
| 5. Skin of White Rats | | | | | | |
| 6. Xenon Flash Lamp | | | | | | |
| 7. Deep Burn Lesions | | | | | | |
| 8. Vascular Thrombosis | | | | | | |
| 9. Tissues - Injury | | | | | | |