cl

CYTOLOGICAL ALTERATION IN THE RAT STOMACH POSTBURN

by

CURTIS NORMAN HARRIS

B. Sc., Oregon State University

M.D., Loma Linda University

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in the Department of

SURGERY

We accept this thesis as conforming to the

required standard

THE UNIVERSITY OF BRITISH COLUMBIA

September, 1972

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Surgery

The University of British Columbia
Vancouver 8, Canada

Date 28 Sept 72

ABSTRACT

Gastric mucosal erosions were induced in the glandular stomach of rats by scalding. The incorporation of Thymidine-methyl^{3}-H into desoxyribonucleic acid was used to determine changes in gastric epithelial cell proliferating ability. Total desoxyribonucleic acid per milligram of gastric tissue was also determined. Sampling was done at twenty-four hours, seven days, and fifteen days postburn.

Eighty-nine point two percent of rats with a standard 26.5 $\pm$ 2% scald burn had developed gastric mucosal erosions by twenty-four hours postburn. Seventeen point eight percent of burned rats had erosions by seven days and the incidence rose to 46.4% by fifteen days postburn. Ten point three percent of control rats in all sampling periods developed erosions.

The total desoxyribonucleic acid in the gastric samples did not change significantly in any treatment period nor was it changed by treatment. Uptake of thymidine-methyl^{3}-H was depressed twenty-four hours postburn and renewed so through seven days postburn. The results at the fifteen day sampling were inconclusive.

By light microscope, the gastric surface epithelium was lifted from the lamina propria and at times there was complete denudation of this cell layer.

The rat is a satisfactory animal model for gross study of mucosal erosions to at least fifteen day postburn. Because of eschar cannibalization inducing variable secretory status, the rat model was not suitable for thymidine uptake studies past seven days postburn.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

Page

INTRODUCTION

In 1842 Thomas Curling (9) presented ten cases of duodenal ulceration associated with burns before the Medical-Surgical Society of London. Since that time the lesion which bears his name has been expanded to include gastric ulcerations as well as duodenal. Other traumatic and disease states have also been associated with gastrointestinal ulcerations; e.g. intracranial lesion (10), trauma (18), coronary occlusion (30), cor pulmonale (15), and these are now collectively termed "STRESS ULCER."

In 1965 Sevitt (44) reported an incidence of twenty-two percent gastric and duodenal ulcerations in two-hundred and ninety-one patients autopsied following burns. In 1970, Pruitt and his group from Brooke Army Hospital reported an overall incidence of 11.7 percent "Curling's Ulcer" with approximately 19 percent of those dying of a cause directly related to the ulcer or an operation thereon (38). This complication is a significant problem in burns as well as other forms of trauma.

The cause or causes of these ulcerations is not known. Following Davenport's experiments in 1964 (13, 12) there has been an increased impetus to determine what defect occurs in the gastric mucosal barrier during episodes of stress. Increasing evidence indicates that there may not be one cause and each different type of stress may have a different elliciting mechanism.

The mucous layer (35, 37, 2, 25, 8), gastric acid (36, 29, 31, 4), gastric epithelial cell (29, 49, 40, 26) and electrolyte flux (11, 13, 12, 23, 31) have all been subjected to study clinically and experimentally. In the experimental animal the stress has been most often induced by restraint (29, 31) with the duration therefore being short.

This experiment was undertaken to study changes in the proliferation of the gastric epithelial cell following burn induced stress in the rat. As the clinical burn is a chronic illness, the duration of the study was extended beyond the usual short 24-48 hour treatment.

METHODS OF EXPERIMENTATION

Animals

White male and female Wistar rats weighing from 175 grams to 275 grams were randomly paired. These pairs were then divided into three treatment groups of twenty-eight pairs. The animals were placed in individual cages and given a standard laboratory diet and water ad libitum. Food was withdrawn eighteen hours prior to treatment or sacrifice. Water was allowed ad libitum at all times.

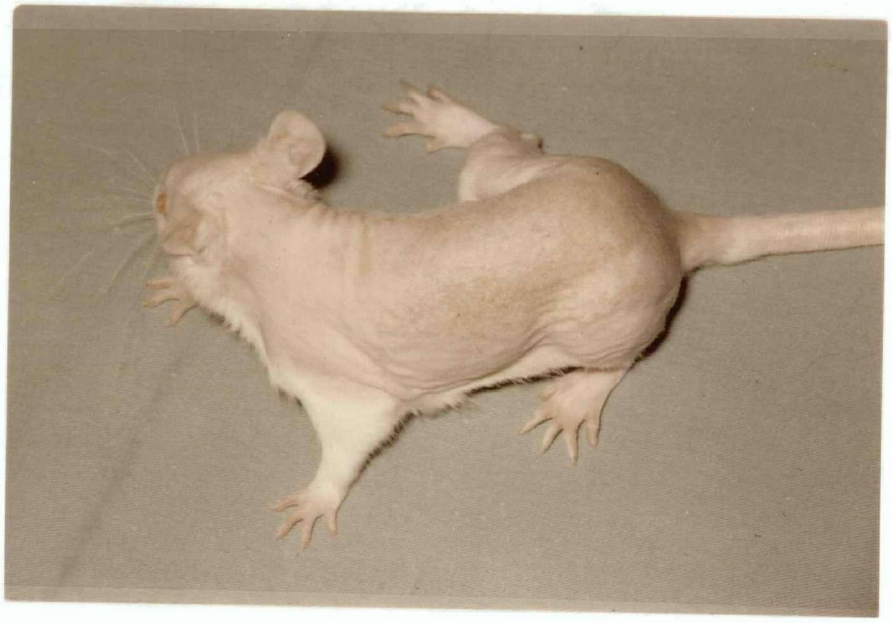
Preparation for Treatment

The rats were anesthetized with Sodium Pentobarbital, 5 milligrams per kilogram intraperitoneally. The backs were clipped with standard animal clippers from behind the ears to the tail and well down on the flanks. "NEET", a commercially available depilatory was next applied to the clipped area, allowed to remain a few minutes and then washed off leaving the skin bare of hair. The animals were then dried with a towel and are shown for treatment in Figure I. Those rats randomly selected to be controls received 20 millilitres of physiological saline intraperitoneally and were returned to their cages.

Method of Treatment

A standard 26.5 $\pm$ 2 percent full thickness burn was produced

Figure I: Clipped and Epilated Wistar rat prior to treatment.



on the back of the rat by scalding. This was accomplished by using the weight immersion principle and a machine originally developed by Bailey, Lewis and Blocker (1) and extensively modified by Courtemanche (6). The temperature of the water was maintained at 88° Centigrade and the duration of treatment was twenty seconds. Following burning the rats were dried, given 20 millilitres of physiological saline intraperitoneally and returned to their cages.

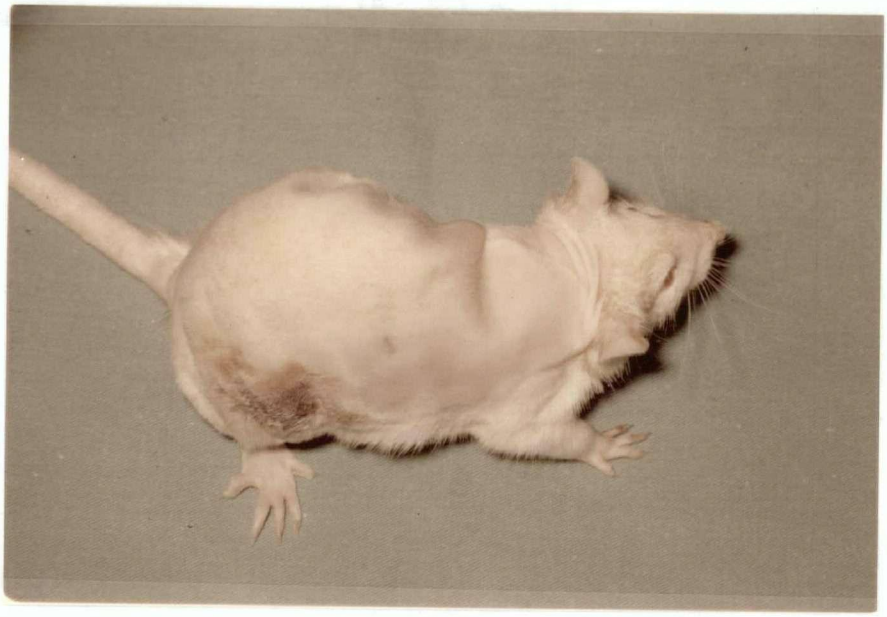
The pairs of rats were sacrificed at twenty-four hours, seven days and fifteen days postburn, and the appearance of the burned rats is shown in Figure II on page 8, Figure III on page 8, and Figure IV on page 10 respectively. Twenty-eight pairs of rats were in each treatment-day group. Following an eighteen hour fast, except for water, they were anesthetized with ether and the abdomen opened. The esophagus and pylorus were ligated with black silk and the stomach excised and placed immediately in ice cold 0.9 Normal saline. The animals were then killed by incising the aorta or femoral artery and allowing exsanguination. Animals dying before sacrifice were discarded.

Gastric Lesions

The excised stomachs were opened along the greater curvature and the glandular stomach examined with a 10 x power dissecting microscope for mucosal erosions. The presence or absence of lesions was recorded and the significance determined by using the Pearson Chi Square test with continuity correction (17).

Figure II: White Wistar rat twenty-four hours postburn.

Figure III: White Wistar rat seven days postburn.



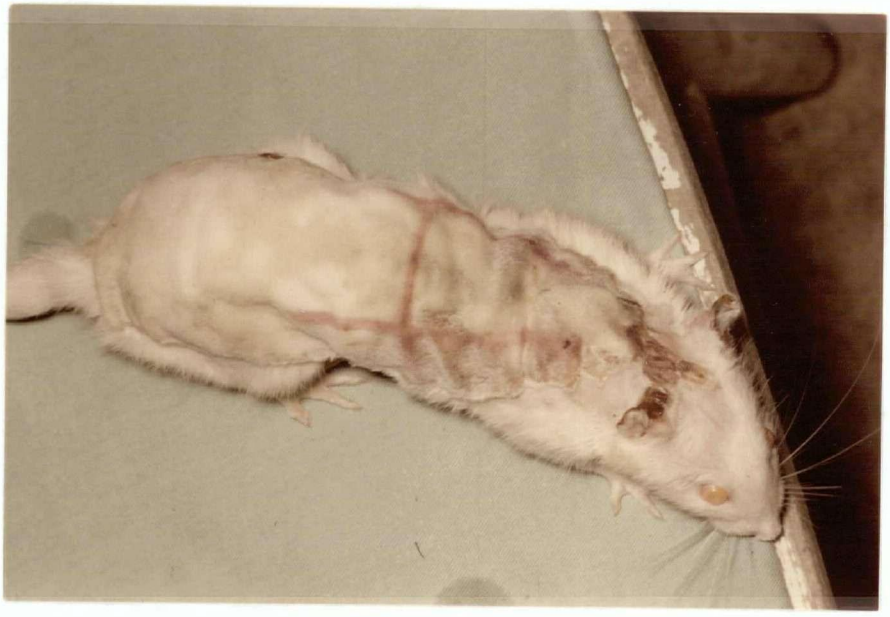


Figure IV: White Wistar rat fifteen days postburn.

Statistics were computed to test treatment effect, batch and day effects using the University of British Columbia Computing Centre's ANOVAR system (17).

Histology

Sections of the glandular stomach were removed for histological examination by hematoxylin and eosin staining (14).

Nucleic Acid Studies

The incorporation of Thymidine-methyl^3-H (TdR^3-H, 20 c/m Swartz-Mann) into the deoxyribonucleic acid of the gastric epithelial cell was measured in control and burned rats. At the completion of the treatment period each rat was injected intraperitoneally with 250 µc of TdR^3-H per kilogram of body weight. Treatment was continued for ninety minutes before sacrifice.

Full thickness specimens of glandular stomach of approximately equal size were obtained using a #3 cork borer. Each specimen was weighed and immediately placed in ice-cold 0.01 molar Tris Buffer for total DNA analysis (29) or Hyamine hydroxide for TdR^3-H uptake analysis (39). An attempt was made not to include erosions in the specimens.

Total DNA was extracted by a method described by Ludwig and Lipkin (29). Two full thickness specimen cores were homogenized with a Teflon pestle in 3 millilitres of ice-cold 0.01 molar Tris Buffer. The acid insoluble material was precipitated by adding

one millilitre of cold 2N perchloric acid and the precipitate then washed twice with two millilitres of 75, 95 and 100 percent alcohol, a one to one mixture of alcohol-ether and ether. The remaining sample was allowed to dry overnight. The next day these samples were hydrolyzed in one millilitre of 0.5 N perchloric acid.

The diphenylamine procedure of Seibert (43, 5) was used for the final colorimetric determination of total DNA. One volume of the hydrolized material was added to two volumes of diphenylamine solution and the mixture heated at 100° C for ten minutes. The absorption curve was read on a Perkin-Elmer spectrophotometer at 650 μ and 595 μ (5). Standardization was with highly polymerized calf thymus deoxyribonucleic acid (Sigma Chemical Company). Results were recorded as micrograms of DNA per milligram of tissue.

One full thickness core of glandular stomach was placed in a scintillation vial with one millilitre of Hyamine Hydroxide and digested at 50° C for 48 hours (47, 39, 50). After digestion the sample was decolorized with six drops of 30 percent hydrogen peroxide (19) and acidified to pH 5 with four to six drops of glacial acetic acid (20). Fifteen millilitres of Aquasol Scintillation fluid (New England Nuclear) were then added, mixed, and the solution counted for ten minutes in the Picker Liquid Scintillation Counter. Results were recorded as counts per minute per milligram of tissue. Final figures were subjected to two way analysis-of-variance for count rate and for total DNA.

RESULTS

Incidence of Gastric Erosions

In Figure V, frequency of erosions of the glandular stomach of burned and unburned rats is shown. Lesions developed in 10.7 (3 of 28) per cent of unburned rats in each of the three treatment periods. Eighty-nine point two percent (25 of 28) of burned rats had developed erosions by twenty-four hours post treatment. By day seven the number of burned rats with erosions had declined to 17.8 percent (5 of 28). The frequency of lesions then increased again in the treatment group at day fifteen to 46.4 percent (13 of 28).

The significance of these results as analyzed by the Pearson Chi Square test is also shown in Figure V. At one day $p \leq 0.0001$ and at fifteen days $p=0.008$. The frequency of gastric ulcerations in burned versus nonburned rats was not of statistical significance at seven days post treatment ($p=0.703$). The maximum number of erosions occurred in the burned animals during the first twenty-four hour treatment period.

Histology, Gross and Microscopic

Gross Specimens

In Figure VI, page 17, are shown the gross characteristics of the opened stomach in an untreated rat. The pink glandular

Figure V: Percentage of animals developing gastric lesions during treatment periods.

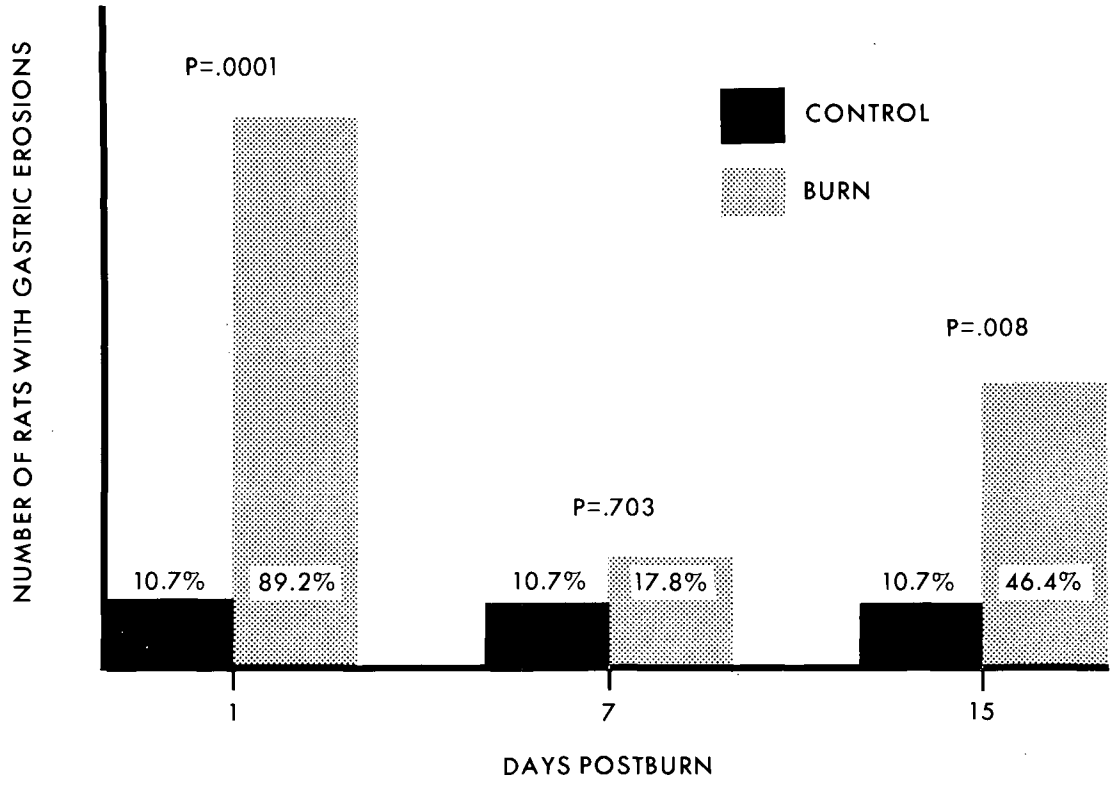

NUMBER OF RATS WITH GASTRIC EROSIONS
P=.0001
CONTROL
BURN
P=.008
P=.703
10.7%
89.2%
10.7%
17.8%
10.7%
46.4%
1
7
15
DAYS POSTBURN

Figure VI: Opened stomach from unburned rat with no mucosal lesions.

Figure VII: Multiple mucosal erosions in stomach of rat twenty-four hours postburn.

portion of the stomach is clearly without lesions. In contrast is the stomach from a burned rat shown in Figure VII, page 17. Multiple erosions of various sizes are present on the glandular portion of this specimen. Some treated animals seemed more likely to develop multiple ulcerations than others, given the same amount of stress. Multiple lesions were more frequent overall than single lesions as shown in Table I. The frequency of multiple lesions did not increase with duration of treatment in this experiment in contrast to the increasing numbers of lesions with duration of treatment shown by Ludwig and Lipkin (29) in their study of restraint stressed guinea pigs. Multiple erosions were not seen in the untreated animals.

Microscopic Specimens

An hematoxylin and eosin preparation of normal rat skin after clipping and epilation with NEET is shown in Figure VIII, page 21. The epidermis and dermis have been minimally changed by the chemical treatment. Figure IX, page 21, is a representative section after burn treatment demonstrating the full thickness injury imposed by the burn. Disruption of the epidermis with coagulation necrosis of the underlying dermis and subcutaneous tissue are readily discernible.

The glandular stomach of the rat is normally covered with columnar epithelial cells with mucous neck cells, parietal and zymogenic cells lining the gastric pits as shown in Figure X, page 24. Changes following treatment were mainly confined to the surface

TABLE I

Frequency Distribution of Number of Erosions per Stomach During Treatment Periods.

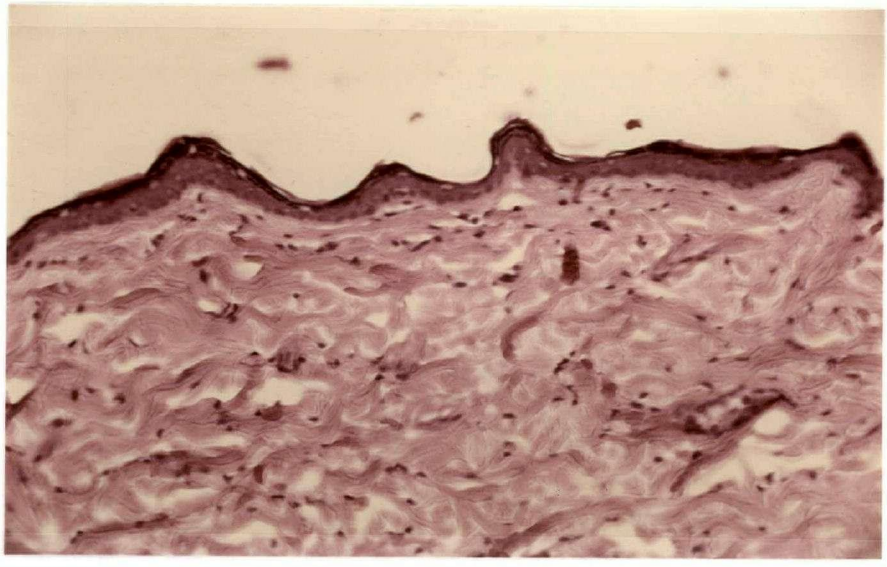
Number of Stomachs

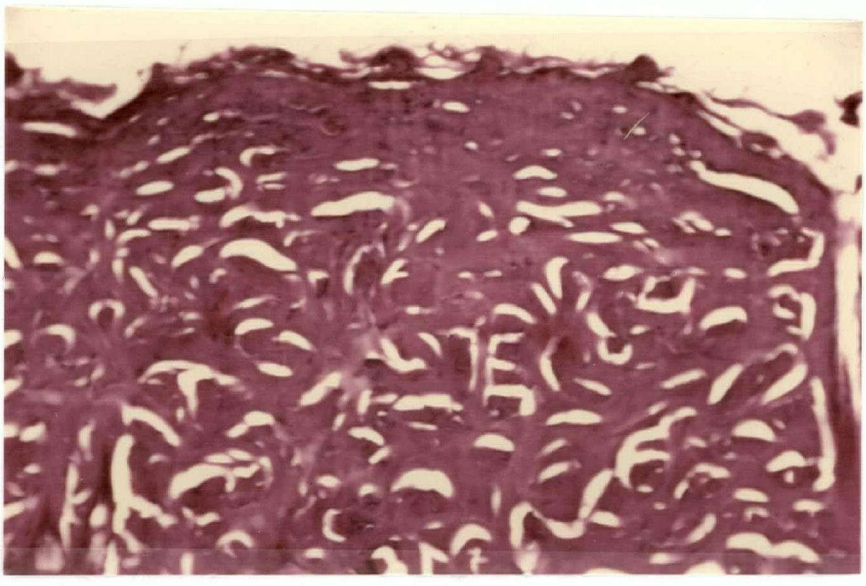
Number of erosions per stomach.	Day 1	Day 7	Day 15
1	2	2	5
2-3	9	1	5
4+	14	2	3
	*92	60	61.9

* Percent of total stomachs with erosions in which lesions were multiple.

Figure VIII: Unburned rat skin after clipping and epilation (from original magnification x120).

Figure IX: Full thickness injury to rat skin after burn treatment at 88°C for twenty seconds (from original magnification x 120).





epithelium and sub-epithelial areas. Many histological sections were similar to Figure XI, page 24, in showing large patches of surface epithelium lifted from the lamina propria. More marked damage is seen in some other areas (Figure XII, page 26), where there was complete destruction of the surface epithelium. Both of these histological changes were more frequent twenty-four hours postburn than later treatment periods. Non-burned rats rarely exhibited subepithelial vacuolization and never denudation.

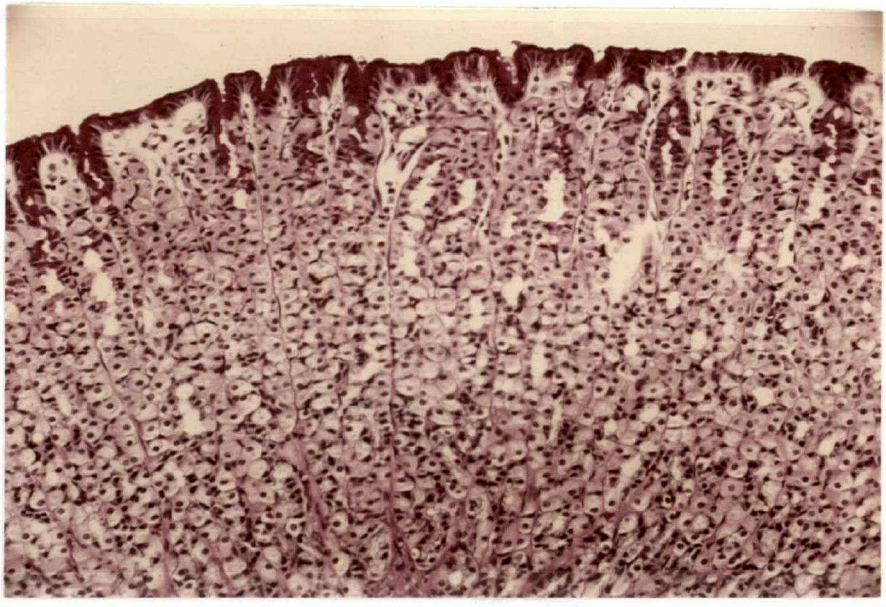
Incorporation of Thymidine-methyl-^{3}H (TdR^3-H)

The surface epithelium of the rat stomach is a renewing cell population (35) and does so at a relatively constant rate in the unstressed animal (34). The rate of regeneration is not significantly changed by the age of the animal (21) but the secretory rate is a major factor. Hunt demonstrated a marked increase in the gastric epithelial cell mitotic activity in the secreting rat over the fasting animal (22), thus, the importance of food withdrawal eighteen hours before treatment or sacrifice in this study.

Each cell nucleus contains desoxyribonucleic acid (DNA). This consists of two long polynucleotide chains made up of pyrimidine bases, phosphoric acid and pentose sugars. With cell duplication the specific pyrimidine base Thymine is taken up from the circulating pool and it is at this point TdR^3-H can be inserted as a label (42) to estimate changes in cell populations. A close relationship exists

Figure X: Intact surface epithelium of gastric mucosa from unburned rat (from original magnification x 120).

Figure XI: Extensive sub epithelial vacuolization of gastric mucosa from a burned rat (from original magnification x120).



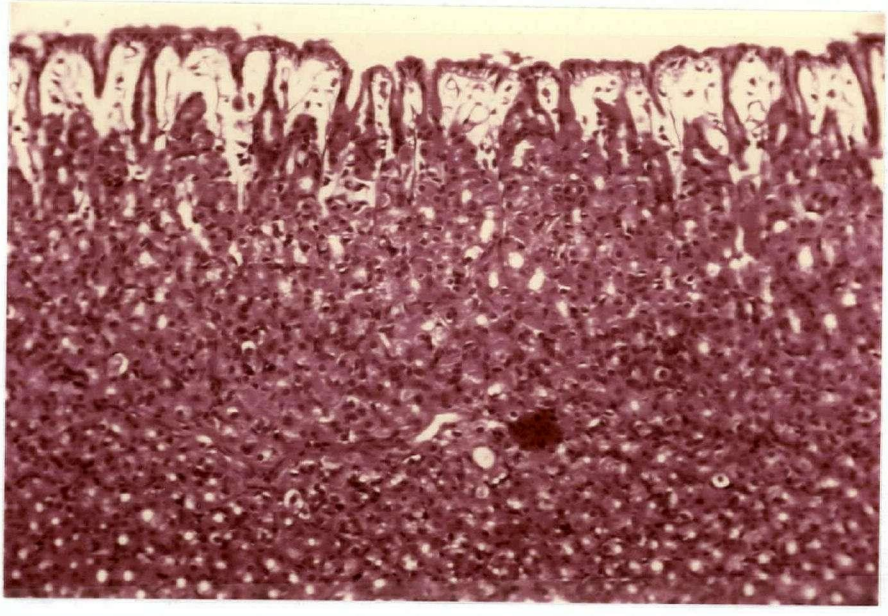
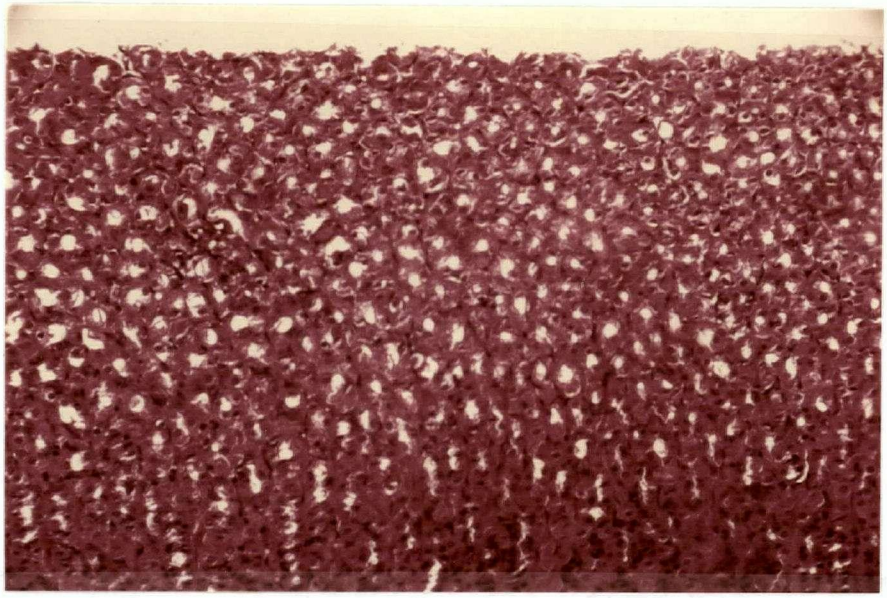


Figure XII: Absence of surface epithelium of gastric mucosa from a burned rat (from original magnification x120).



between the uptake of this labeled Thymidine and mitosis (35). Each species has a constant amount of DNA per nucleus (48). This serves as a baseline which in conjunction with TdR^3-H uptake by the gastric epithelial cell allows an accurate estimation of changes in the proliferation ability of these cells.

The specific activity of TdR^3-H is shown in Figure XIII. There is a significant decrease in uptake in burned rats ($p < 0.0001$) at twenty-four hours post treatment. This reduction in uptake continued through day seven ($p = .0015$). On the fifteenth postburn day there was an apparent increase in uptake in the treated group over the untreated animals. However, in subjecting this to analysis of variance this increase was not statistically significant ($p=0.6591$).

Total Cellular Desoxyribonucleic Acid

In Figure XIV, page 31, are shown the relationships of total DNA in the nonburned and burned rats during the fifteen day treatment period. The range was 13 to 56 micrograms of DNA per milligram of glandular stomach with a mean of 38.579 micrograms per milligram in untreated animals and a mean of 40.436 micrograms per milligram in the treated ones. Computing the statistical significance by the analysis of variance resulted in $p=0.0903$ at 24 hours, $p=0.7010$ at seven days and $p=0.4376$ at fifteen days.

Figure XIII: Specific activity of Desoxyribonucleic Acid following injection of Thymidine-methyl-^{3}H in the glandular stomach in unburned rats and rats 24 hours, 7 days and 15 days postburn.

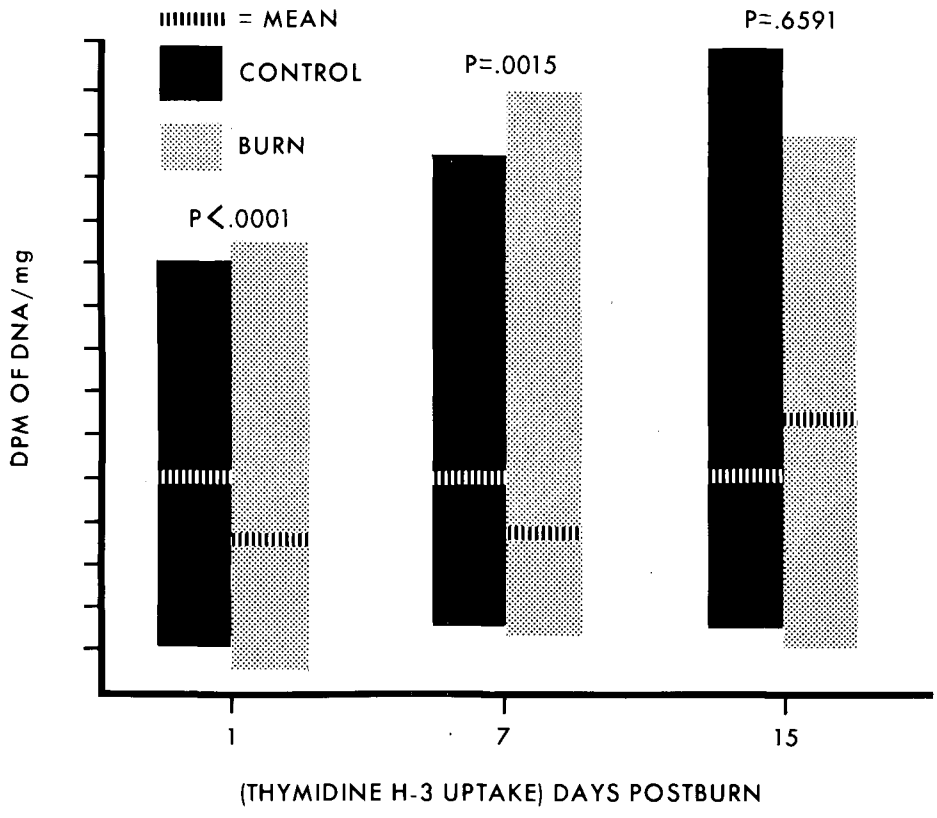
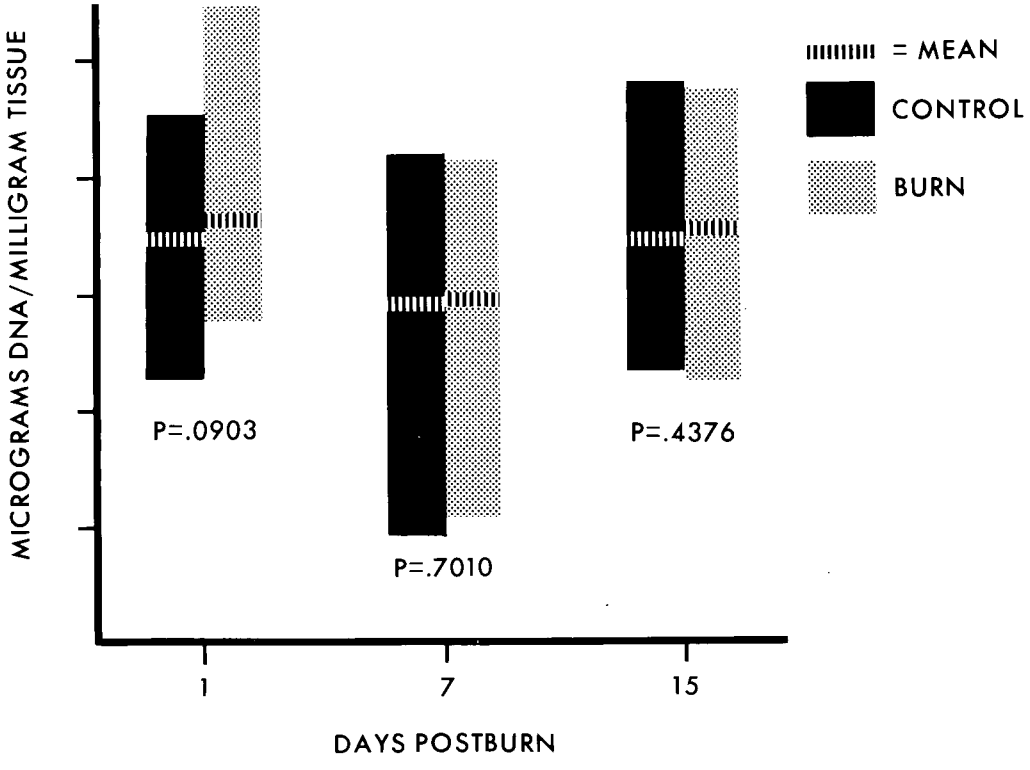

= MEAN
CONTROL
BURN
P<.0001
P=.0015
P=.6591
DPM OF DNA/mg
1
7
15
(THYMIDINE H-3 UPTAKE) DAYS POSTBURN

Figure XIV: Total Desoxyribonucleic Acid content of tissue samples from glandular stomachs of unburned and burned rats after 24 hours, 7 day and 15 day treatment periods.


MICROGRAMS DNA/MILLIGRAM TISSUE
= MEAN
CONTROL
BURN
P=.0903
P=.7010
P=.4376
1
7
15
DAYS POSTBURN

SUMMARY AND CONCLUSIONS

Summary:

This experiment has demonstrated the feasibility of using the standard burned rat to study burn induced gastric changes to fifteen days postburn. Gross and microscopic changes are easily followed.

The incidence of mucosal lesions during the first twenty-four hours (89.2%) was similar to other reports (31, 41, 16, 3). A biphasic distribution of erosions with peaks at one day and fifteen days postburn was observed. This resembles the results reported by Sevitt (44) in his autopsy study of burned humans. The mechanism of this is not known but he suggested that the initial lesions were as a direct result of the injury with subsequent healing, and then reulceration. Daily sampling of burned animals throughout the treatment period would be helpful in confirming this distribution.

The incidence of mucosal lesions in control animals was higher (10.7%) than most studies. The anesthesia, clipping, epilating and washing of these animals apparently produced a significant amount of stress.

Variability of the rats in response to stress was also observed. Genetic factors (45) and environmental factors such as housing and diet (24, 46) have been implicated. All rats in this study were of

similar breeding and received identical pre-stress treatment thus minimizing any of these differences.

Morphological study demonstrated formation of a subepithelial space. This progressive lifting of the surface epithelium resulted in denudation and probably subsequent digestion of the lamina propria by intra luminal enzymes. These are similar to observations reported by Chiu, McArdle and Brown in a study of low flow rates and intestinal epithelium (7). In their experiment the rats were fasted twenty-four hours after scalding. This fluid deprivation was found to increase the incidence of gastric erosion. They postulated that this increase was secondary to a low flow state. In this study rats were allowed ad libitum water before and after burning. In addition, intraperitoneal saline was given immediately after treatment. A high incidence of mucosal erosions still occurred. This would seem to refute Chiu's postulation but may mean only that a low flow state occurred in spite of the fluid intake, the fluid intake was inadequate for the degree of trauma or other unknown mechanisms were in force.

The incorporation of thymidine into desoxyribonucleic acid was depressed at twenty-four hours postburn and remained low through seven days postburn. By fifteen days postburn an overall increase in thymidine uptake appeared to be present in the burned animals. This situation can probably be attributed to the variable secretory rate in this group and which is reflected in the wide range of counts (Appendix A). It was not possible to maintain a fasting state in

some of these animals as is required for basal mitotic activity (22) because many rats would cannibalize their own eschar. This increases secretory rate and thus thymidine uptake. No satisfactory method to prevent this was devised other than to substitute a non-carnivorous animal such as the guinea pig.

Total DNA content remained unchanged and in association with the uptake studies indicates reduction in thymidine incorporation into DNA and thus decreased DNA synthesis and cell proliferation to day seven postburn. Continued depression of proliferation likely occurs but in view of the above cited difficulties remains unproven.

Conclusions:

1. The rat is a satisfactory animal for gross and microscopic study of gastric erosions up to fifteen days postburn.

2. Gastric mucosal cell proliferation is significantly depressed one day to seven days postburn as measured by thymidine-methyl^{3}-H. Eschar cannibalization is the probable cause of variable results at fifteen days postburn.

3. The total DNA content of the stomach mucosa is not significantly changed within fifteen days postburn despite the severe injury and resulting debility.

4. The exact etiology of mucosal erosions in burns remains unknown but the decreased ability of the surface epithelium to renew adequately seems to be a contributing factor.

BIBLIOGRAPHY

1. Bailey, B.N., S.R. Lewis and T.G. Blocken, "Standardization of Experimental Burns in the Laboratory Rat", Texas Reports on Biology & Medicine, 20: 20, 1962.

2. Ballinger, W.F. & L. Wise, "Gastric Mucous; Quantitative and Qualitative Studies in Humans & Dogs", British Journal of Surgery, Vol. 56: 701, 1969.

3. Brodie, D.A., "Experimental Peptic Ulcer", Gastroenterology, Vol. 55: 125, 1968.

4. Brodie, D.A., "Effect of Restraint Stress on Gastric Acidity in the Rat", American Journal of Physiology, Vol. 202: 812, 1962.

5. Chargoff, E., & S.N. Davidson, ed., L. Dische, "Color Reactions of Nucleic Acid Components", The Nucleic Acids, Vol. I, p. 285, 1955.

6. Courtemanche, A.D., "A Controlled Burning Apparatus Based on the Weight Immersion Principle", British Journal of Plastic Surgery, Vol. 19: 23, 1966.

7. Chiu, C.J., A.H. McArdle, & R.A. Brown, "Intestinal Mucosal Lesion in Low Flow States: I. A Morphological, Hemodynamic and Metabolic Reappraisal", Archives of Surgery, Vol. 101: 478, 1970.

8. Chiu, C.J., A.H. McArdle, & R.A. Brown, "Gastric Mucosal Changes Following Burns in Rats", Archives of Surgery, Vol. 103: 147, 1971.

9. Curling, T.B., "On Ulceration of the Duodenum in Cases of Burn", Medico-Chirurgical Transactions, Vol. 25: 260-281, 1842.

10. Cushing, H., "Peptic Ulcers and the Interbrain", Surgery, Gynecology & Obstetrics, Vol. 55: 1-33, 1932.

11. Davenport, H.W., "Salicylate Damage to the Gastric Mucosal Barrier", New England Journal of Medicine, Vol. 276: 1307, 1970.

12. Davenport, H.W., "Gastric Mucosal Injury by Fatty & Acetylsalicylic Acids", Gastroenterology, Vol. 46: 245, 1964.

13. Davenport, H.W., "Functional Significance of Gastric Mucosal Barrier to Sodium", Gastroenterology, Vol. 47: 142, 1964.

14. Drury, R.A.B., & E.A. Wallington, Carleton's Histological Technique. 1967.

15. Flint, F., & A.J.N. Warrack, "Acute Peptic Ulceration in Emphysema", Lancet, 2: 178, 1958.

16. Foss, D.L., L.S. Stavney, T. Havaguchi, H.N. Harkins, L.M. Nyhus, "Pathophysiologic & Therapeutic Considerations of Curling's Ulcer in the Rat", Journal of American Medical Association, Vol. 187: 592, 1964.

17. Glick, Ned. Personal communication. 1972.

18. Grosz, C.R., & K. Wu, "Stress Ulcer: A Survey of Experience in a Large General Hospital", Surgery, Vol. 61: 853-857, 1967.

19. Herberg, R.J., "Determination of Carbon 14 & Tritium in Blood and Other Whole Tissues", Analytical Chemistry, Vol. 32: 43, 1960.

20. Herberg, R., "Phosphorescence in Liquid Scintillation Counting of Proteins", Science, 128: 199, 1958.

21. Hunt, Thomas, "Regeneration of the Gastric Mucosa of the Rat", Anatomical Record, Vol. 131: 193, 1958.

22. Hunt, Thomas, "Mitotic Activity in the Gastric Mucosa of the Rat after Fasting and Refeeding", Anatomical Record, Vol. 127: 539, 1957.

23. Ivey, Kevin, "Gastric Mucosal Barrier", Gastroenterology, Vol. 61: 247, 1971.

24. Kim, Y.S., & J.P. Lambooy, "Riboflavin Deficiency and Gastric Ulcer Production in the Rat: A Procedure for the Study of Susceptibility to Stress Induced Gastric Ulcers", Journal of Nutrition, Vol. 91: 183, 1967.

25. Kowalewski, K., G.G. Chmura, Chir Dent, J. Scheir, "Experimental Deficiency of Gastric 'Mucous Barrier'", American Journal of Digestive Diseases, Vol. 14: 733, 1969.

26. Lahtiharju, A., & T. Rytomaz, "DNA Synthesis in Fore and Glandular Stomach & Skin in Mice after Non-Specific Stress", Experimental Cell Research, Vol. 46: 593, 1967.

27. Lucas, C.E., C. Sugawa, W. Friend, A.J. Walt, "Therapeutic Implications of Disturbed Gastric Physiology in Patients with Stress Ulceration," American Journal of Surgery, Vol. 123: 25, 1972.

28. Lucas, C.E., C. Sugawa, J. Riddle, F. Rector, B. Rosenberg, A.J. Walt, "Natural History & Surgical Dilemma of 'Stress' Gastric Bleeding," Archives of Surgery, Vol. 102: 266.

29. Ludwig, William M., & Martin Lipkin, "Biochemical and Cytological Alterations in Gastric Mucosa of Guinea Pigs under Restraint Stress," Gastroenterology, Vol. 56: 895, 1969.

30. Mears, F.B., "Autopsy Survey of Peptic Ulcer Associated with Other Diseases, A Review of Related Etiological Factors Concerned," Surgery, Vol. 34: 640, 1953.

31. Menguy, Rene, "Effects of Restraint Stress on Gastric Secretion in the Rat," American Journal of Digestive Diseases, Vol. 5: 911, 1960.

32. Menguy, R.B., Y.F. Masters, "The Effect of Cortisone on Mucoprotein Secretion of the Gastric Antrum," Surgery, Vol. 54: 19, 1963.

33. Menguy, R.B., & A.E. Thompson, "Regulation of Secretion of Mucous From the Gastric Antrum," Annals of New York Academy of Science, Vol. 140: 797, 1967.

34. Messier, B., "Radioautographic Evidence of the Renewal of Mucous Cells in the Gastric Mucosa of the Rat," Anatomical Record, Vol. 136: 242, 1960.

35. Messier, B., & C.P. LeBlond, "Cell Proliferation & Migration as Revealed by Radioautography after Injection of Thymidine H^3 into Male Rats & Mice," American Journal of Anatomy, Vol. 106: 247, 1960.

36. O'Neill, James A., Jr., "The Influence of Thermal Burns on Gastric Acid Secretion," Surgery, Vol. 67: 267, 1970.

37. Overholt, B.F., & H.M. Pollard, "Acid Diffusion into the Human Gastric Mucosa," Gastroenterology, Vol. 54: 182, 1968.

38. Pruitt, Basil A., Jr., F.D. Foley, & John A. Moncrief, "Curling's Ulcer: A Clinico-Pathological Study of 323 Cases," Annals of Surgery, Vol. 172: 523, 1970.

39. Rapkin, Edward, "Hydroxide of Hyamine 10-X," Packard Technical Bulletin, #3: 1, June 1961.

40. Rasanen, Tom, "Fluctuations in the Mitotic Frequency of the Glandular Stomach & Intestine of the Rat Under Influence of ACTH, Glucocorticoids, Stress and Heparin."

41. Raulston, G.L., & H.L. Walker, "Effect of Adrenalectomy on Curling's Ulcer Formation in the Burned Rat," Abstract, Research in Biomedical Sciences, No. 3, A061102 B71R-01. U.S. Army Institute of Surgical Research. Brooke Army Medical Center, 1970.

42. Reichard, P., & B. Estborn, "Utilization of Deoxyribonides in the Synthesis of Polynucleotides," Journal of Biological Chemistry, Vol. 188: 839, 1951.

43. Seibert, F.B., "Removal of the Impurities, Nucleic Acid & Polysaccharide From Tuberculin Protein," Journal of Biological Chemistry, Vol. 133: 593, 1940.

44. Sevitt, Simon, "Duodenal & Gastric Ulceration after Burning," British Journal of Surgery, Vol. 54: 32, 1967.

45. Sines, Jo., "Selective Breeding for Development of Stomach Lesions Following Stress in the Rat," Journal of Comparative Physiological Psychology, Vol. 52: 615, 1959.

46. Sines, Jo., "Pre-Stress Sensory Input as a non-Pharmacologic Method for Controlling Restraint-ulcer Susceptibility," Journal of Psychological Research, Vol. 8: 399, 1965.

47. Vaughn, Martha, Daniel Steinberg, & Jane Logan, "Liquid Scintillation Counting of C_{14} & H^3 Labeled Amino Acids & Proteins," Science, Vol. 126: 446, 1957.

48. Vendrely, R., & C. Vendrely, "La Teneur du noyau cellulaire en acide désoxribonucléique à travers les organes, les individus et les espèces animales," Experientia, Vol. 4: 434, 1948.

49. Young, Kim S., Robert Kerr, Martin Lipkin, "Cell Proliferation During Development of Stress Erosions in Mouse Stomach," Nature, 215: 1180, 1967.

50. "Protosol the Superior Solubilizer," New England Nuclear Bulletin, Boston, 1972.

APPENDIX

TABLE II

Thymidine-methyl^{3}-H uptake counts and total cellular Desoxyribonucleic Acid for twenty-four hour treated and untreated rats.

	TdR^{3}-H Scintillation Counts/Milligrams Tissue/Minute		Total Micrograms DNA/Milligram Tissue	
	Control Rat	Burned Rat	Control Rat	Burned Rat
1.	19.240	12.735	.324	.274
2.	18.639	9.94	.295	.310
3.	25.116	13.292	.370	.390
4.	21.165	15.529	.281	.408
5.	15.854	9.48	.401	.522
6.	17.500	12.491	.436	.428
7.	186.790	165.623	.310	.326
8.	182.117	183.015	.232	.322
9.	201.962	159.355	.387	.342
10.	187.077	109.434	.330	.308
11.	160.425	151.671	.309	.366
12.	171.035	188.338	.330	.460
13.	215.700	209.102	.364	.317
14.	193.954	146.967	.368	.360
15.	169.251	148.147	.282	.406
16.	164.159	153.762	.290	.324
17.	63.960	43.627	.410	.420
18.	61.242	35.257	.422	.406
19.	114.562	37.162	.456	.543
20.	54.661	34.600	.278	.409
21.	57.710	31.682	.365	.356
22.	80.823	30.407	.328	.276
23.	42.892	11.761	.377	.409
24.	84.560	19.406	.337	.260
25.	77.394	17.375	.320	.327
26.	121.310	11.511	.336	.322
27.	48.367	29.050	.373	.420
28.	54.327	18.208	.326	.290

TABLE III

Thymidine-methyl^{3}-H uptake counts and total cellular Desoxyribonucleic Acid for seven days treated and untreated rats

	TdR^{3}-H Scintillation Counts/Milligrams Tissue/Minute		Total Micrograms DNA/Milligram Tissue	
	Control Rat	Burned Rat	Control Rat	Burned Rat
1.	205.907	192.939	.401	.321
2.	170.249	145.372	.314	.271
3.	197.106	136.891	.362	.255
4.	254.672	135.936	.418	.408
5.	134.905	141.183	.333	.353
6.	256.275	155.150	.263	.342
7.	217.945	277.377	.283	.382
8.	213.464	254.475	.328	.336
9.	221.522	231.147	.300	.318
10.	276.131	216.909	.326	.333
11.	236.823	227.287	.361	.330
12.	183.347	180.047	.340	.317
13.	155.022	152.777	.304	.297
14.	216.861	151.064	.408	.362
15.	172.224	158.746	.272	.340
16.	170.665	191.603	.297	.240
17.	251.455	179.528	.267	.311
18.	145.328	129.098	.246	.277
19.	71.068	28.692	.155	.159
20.	48.733	27.598	.174	.228
21.	30.465	28.800	.281	.241
22.	36.313	28.989	.266	.294
23.	64.156	28.787	.089	.155
24.	44.785	36.143	.101	.106
25.	44.860	15.066	.276	.318
26.	52.347	23.934	.276	.336
27.	83.788	28.450	.327	.325
28.	103.061	36.802	.326	.276

TABLE IV

Thymidine-methyl^{3}-H uptake counts and total cellular Desoxyribonucleic Acid for fifteen days treated and untreated rats.

	TdR^{3}-H Scintillation Counts/Milligrams Tissue/Minute		Total Micrograms DNA/Milligram Tissue	
	Control Rat	Burned Rat	Control Rat	Burned Rat
1.	230.211	183.297	.339	.331
2.	323.682	229.735	.291	.259
3.	319.530	463.117	.294	.287
4.	240.009	910.968	.230	.296
5.	289.970	535.234	.295	.363
6.	242.601	111.414	.380	.244
7.	198.861		.285	.224
8.	169.715		.261	.215
9.	38.853	22.177	.316	.338
10.	29.849	54.586	.297	.390
11.	49.106	83.944	.352	.418
12.	72.028	48.413	.319	.323
13.	218.547	238.548	.336	.204
14.	220.316	152.576	.372	.338
15.	194.487	196.343	.371	.340
16.	229.695	152.576	.371	.338
17.	228.085	217.950	.345	.350
18.	257.952	184.940	.385	.440
19.	292.984	223.780	.366	.473
20.	215.026	200.077	.347	.419
21.	202.045	267.961	.370	.471
22.	188.808	145.151	.362	.450
23.	146.461	150.852	.373	.450
24.	222.342	154.588	.391	.383
25.	203.453	163.226	.476	.438
26.	191.102	202.090	.476	.385
27.	311.850	267.494	.342	.400
28.	224.290	240.852	.320	.257