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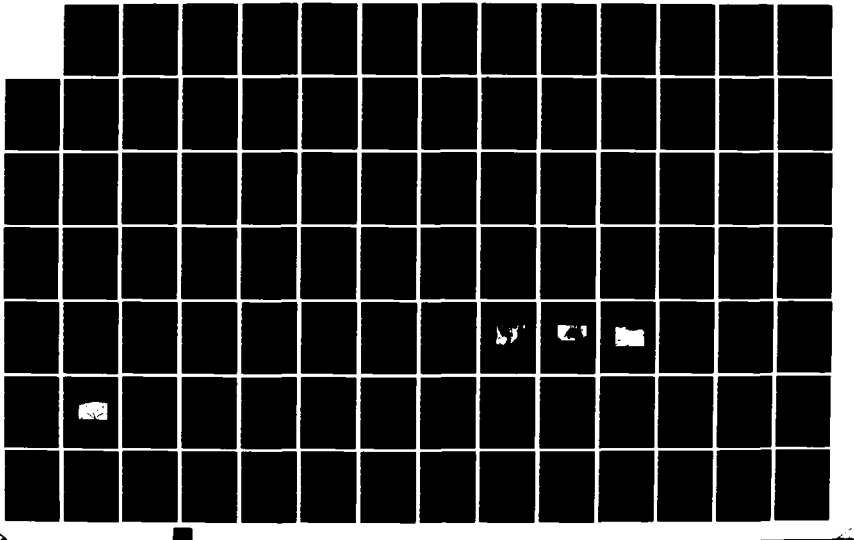

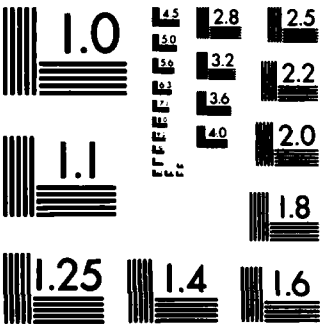

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THESIS ABSTRACT

THE OHIO STATE UNIVERSITY

GRADUATE SCHOOL

ANDREW E. STANYA CAPTAIN USAF DC SUMMER/1983

DEPARTMENT OF PERIODONTICS MASTER OF SCIENCE

TITLE: THE EFFECTS OF VARIOUS LEVELS OF ASCORBIC ACID INTAKE UPON ORAL WOUND HEALING IN GUINEA PIGS

The purpose of this study was to evaluate the effects of varying levels of orally administered ascorbic acid on wound healing in guinea pig oral mucosa. Forty five Murphy/Hartley guinea pigs were randomly placed into four groups and fed an ascorbic acid deficient diet for 2 weeks. Each group of animals then received a daily oral supplement of the following doses of ascorbic acid: 0.5 mg., 5.0 mg., 50 mg., and 250 mg. All animals were weighed twice a week. Twenty eight days later, a standardized wound was made in the premaxilla. On day 36, all animals were sacrificed. Blood samples were evaluated for levels of ascorbic acid. Thirty nine samples showed insignificant levels of vitamin C, 32 of which showed no ascorbic acid. Block sections of the premaxillary wound site were processed for histologic evaluation. Cross sections of the healing wounds were stained with H+E and Masson and were evaluated using a quadratic test grid. Results revealed that varying the levels of ascorbic acid had no significant effect on wound healing at 8 days ($P>.05$). Organ to body weight ratios were calculated and compared. Spleen to body weight ratios were not affected by different ascorbic acid levels ($P>.05$). Low levels of ascorbic acid tended to increase kidney to body weight ratios ($P=.0514$) and high levels caused a significant increase in adrenal gland to body weight ratios ($P<.05$). Varying the levels of ascorbic acid did not affect growth prior to surgical wounding. Increased levels of ascorbic acid enhanced body weight recovery post surgically ($P<.05$).

THESIS ABSTRACT

THE OHIO STATE UNIVERSITY
GRADUATE SCHOOL
(Please type.)

NAME: Andrew E. Stanya QUARTER/YEAR: Summer/1983

DEPARTMENT: Periodontology DEGREE: Master of Science

TITLE OF THESIS: The Effects of Various Levels of Ascorbic Acid Intake upon Oral Wound Healing in Guinea Pigs

Summarize in the space below the purpose and principal conclusions of your thesis.

The purpose of this study was to evaluate the effects of varying levels of orally administered ascorbic acid on wound healing in guinea pig oral mucosa. Forty-five Murphy/Hartley guinea pigs were randomly placed into four groups and fed an ascorbic acid deficient diet for 2 weeks. Each group of animals then received a daily oral supplement of the following doses of ascorbic acid: 0.5 mg., 5.0 mg., 50 mg. and 250 mg. All animals were weighed twice a week. Twenty-eight days later, a standardized wound was made in the premaxilla. On day 36, all animals were sacrificed. Blood samples were evaluated for levels of ascorbic acid. Thirty-nine samples showed insignificant levels of vitamin C 32 of which showed no ascorbic acid. Block sections of the premaxillary wound site were processed for histologic evaluation. Cross sections of the healing wounds were stained with H+E and Masson and were evaluated using a quadratic test grid. Results revealed that varying the levels of ascorbic acid had no significant effect on wound healing at 8 days ($P>.05$). Organ to body weight ratios were calculated and compared. Spleen to body weight ratios were not affected by different ascorbic acid levels ($P>.05$). Low levels of ascorbic acid tended to increase kidney to body weight ratios ($P=.0514$) and high levels caused a significant increase in adrenal gland to body weight ratios ($P<.05$). Varying the levels of ascorbic acid did not affect growth prior to surgical wounding. Increased levels of ascorbic acid enhanced body weight recovery post surgically ($P<.05$).

Charles W. Filter
Adviser's Signature

THE EFFECTS OF VARIOUS LEVELS OF ASCORBIC ACID INTAKE UPON ORAL WOUND HEALING IN GUINEA PIGS

A Thesis

Presented in Partial Fulfillment of the Requirements

for the Degree Master of Science

by

Andrew E. Stanya, B.S., D.D.S.

The Ohio State University

1983

Approved by

Charles W. Salt

Advisor

Department of Periodontology

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INTRODUCTION

Ascorbic acid is an essential water soluble vitamin in humans, primates, fruit-eating bats, red-vented bulbuls, rainbow trout, coho salmon and guinea pigs.[1] They lack the necessary enzyme system for ascorbate synthesis. Continued deprivation of the vitamin leads to scurvy and eventually death.

Ascorbic acid is involved in folic acid activity, iron absorption, maintains integrity of capillary walls, plays some role in stress and is involved in the formation of intercellular ground substance.[2,3,4,5,6] Vitamin C is a reducing agent and sustains various enzyme systems in active states. It is important in the synthesis of collagen and is therefore an important facet in wound healing.

The guinea pig is the animal model of choice to study the effects of ascorbic acid. Guinea pigs develop scorbutic symptoms in 3 to 4 weeks. The condition manifests itself as weight loss, stiffening of the hind legs, hemorrhaging in muscles and joints, reduced collagen and osteoid formation, odontogenic changes and delayed wound healing.[7] Chronic marginal vitamin C deficiency appears as a nonclinical entity and is seen to result in depressed immune response, atherosclerotic changes, alterations in liver cells

and in the autonomic ganglia and defective wound healing.[8,9,10]

In recent years, massive doses of vitamin C have been advocated in humans for cold prevention, improved well being, treatment of cancer and inflammatory periodontal disease.[11,12,13,14] Guinea pig studies have indicated improved wound healing.[15]

The purpose of this study is to evaluate the effects of varying levels of orally administered ascorbic acid on wound healing in guinea pig oral mucosa. Wound healing is to be evaluated histomorphometrically.

LITERATURE REVIEW

Vitamin C was isolated in 1928 by Szent-Gyorgyi from orange and cabbage juice and the adrenal glands of oxen.[16] "Hexuronic acid," as it was called then, was synthesized in 1933 by Reichstein. Due to its antiscorbutic activities, it was labeled ascorbic acid.

Ascorbic acid is a six carbon chain similar in structure to glucose with the empirical formula $C_6H_8O_6$. It is a white, odorless, crystaline substance with a molecular weight of 176.06 daltons. Vitamin C is heat labile and deteriorates in air. L-ascorbic acid is the active form of vitamin C.. Ultraviolet light absorption of L-ascorbic acid occurs at 245 nm. if the pH is less than 1.5. Ascorbic acid is a strong reducing agent and is readily oxidized to dehydroascorbic acid, which is also antiscorbutic.[5] The exact biochemical functions are unknown, but appear to maintain enzymes and their cofactors in an active state, as well as acting as an antioxidant.

Ascorbic acid is involved in a wide variety of metabolic activities including folate, tyrosine, cholesterol and carbohydrate metabolism.[8,15] It also plays a role in mineral activity.[5]

Vitamin C plays an active role in the immune response. Early studies indicated enhanced ability

of guinea pigs to resist tuberculin infection.[17] Polymorphonuclear leukocytes absorb large amounts of vitamin C, especially when migrating into infected areas.[18] Alvares observed impaired polymorphonuclear leukocytes, chemotaxis and phagocytosis in monkeys with subclinical ascorbate deficiency. Increased gingival inflammation and pocketing occurred.[9]

Orally administered ascorbic acid is absorbed in the ileum via an active transport mechanism which is sodium dependent in the guinea pig and man.[19,20,21,22] Studies indicate a 27 to 50% loss in absorption of orally administered doses compared to intravenous and intramuscular administration.[23,24,25] Diarrhea, drugs and the terminal stages of scurvy reduce intestinal absorption.[26,27,28] Blood levels increase significantly after vitamin C administration, especially in leukocytes and platelets. Plasma levels increase to a lesser degree and are used to determine ascorbic acid metabolic turnover rate.[29] Plasma levels can fluctuate significantly due to uptake by tissues and circulating leukocytes.[30,31] Vitamin C concentrates in tissues with increased metabolic demands. These include glands, wound sites, muscle and fatty tissue.[32,33,34,35,36,37,38,39,40]

Ascorbic acid is absorbed most efficiently in small doses.[41,19] Its half life is 2 to 6 days. Once saturation is obtained in the guinea pig, the unused ascorbic acid is oxidized to carbon dioxide and eliminated via the respiratory route.[42,43] Given high doses of vitamin C, the guinea pig excretes it in the urine. Man eliminates ascorbic acid primarily in the urine.

Ascorbic acid is a primary factor in the metabolism of collagen, a major constituent of connective tissue in skin and gingiva. It is important in the hydroxylation of proline and lysine. Hydroxyproline stabilizes the helical structure.[44] Hydroxylysine plays a critical role in cross-linkage formation.[45] Recently, studies have indicated that fibroblasts themselves may be directly stimulated to increase collagen production, possibly by stimulation with Type I procollagen messenger-RNA.[46,47] Wound studies have used collagen production as a basis for evaluating the relationship of ascorbic acid to healing.[4,47,48] Alfano observed increased permeability of oral tissue with decreased vitamin C supplementation. Periodontal tissues are susceptible to permeable toxic products elicited from inflammatory periodontal disease.[50] Kramer found that large doses

of vitamin C stimulated significant collagen production in implanted polyvinyl sponges after 1 week in the guinea pig.[15]

Acute ascorbic acid deficiency has a marked effect on the connective tissues in the guinea pig. In general, there is a decreased production of intercellular substance, edema, degeneration of fibroblasts and breakdown of newly formed reticular and collagen fibers.[51] Hyaluronic acid accumulates and there is decreased incorporation of sulfate into acid mucopolysaccharides.[44,5] There is impaired formation of the basement membrane.[52] Vascular changes include swelling of the endothelial cells, thinning of vessel walls, fatty and granular degeneration, cell separation in walls of larger vessels, capillary fragility and collagen degeneration.[51] Intercellular hemorrhaging occurs, due to decreased intercellular ground substance.[7,52,53,54] Blood vessels are randomly oriented and fewer in number.[52,55]

Osseous changes in ascorbic acid deficiency are related to the lack of bone deposition. Osteoid tissue is not formed even though there is continued proliferation of osteoblasts.[56] Osteoblastic activity decreases.[5] Osteoporosis is seen in long bone production, rather than increased osteoclastic activity.

Irregular junctions occur between bone and cartilage. There is failure to form ground substance, along with subperiosteal hemorrhaging and loss of collagen in bone. Calcium fixation in bones is reduced along with a decrease in calcium:phosphorus ratios.[5] Alkaline phosphatase activity significantly decreases in bone and gingiva while acid phosphatase increases in wounds of deficient animals.[55,60]

Dental tissues require significant levels of ascorbic acid.[35,40] Odontoblasts degenerate and are unable to form dental matrix in a deficient state.[7,56] Dentin becomes porous and pulpal tissues hemorrhage as blood vessels dilatate.[57] The periodontal ligament fibers become less dense as collagen production is decreased.[5] Cementoblasts degenerate and are unable to deposit cementum. Carious lesions increase in deficient guinea pigs.[61]

Acute ascorbic acid deficient conditions are well documented. Suboptimal intake of ascorbic acid may be common in guinea pigs and humans.[62,63,64] Clinically, chronic hypovitaminosis C of chronic subclinical scurvy display no overt scorbutic signs. Ginter maintained adult guinea pigs with 0.5 mg./day of ascorbic acid for 68 days with no weight loss and no overt scorbutic signs. At the time of sacrifice, he observed

enlargement of the spleen and kidneys in the guinea pig.[66,67] Others have observed arteriosclerotic changes in the aorta and impaired polymorphonuclear leucocyte chemotaxis.[8,9] Wound healing was delayed and inadequate.[68,34,10]

Massive doses of ascorbic acid have been advocated for improved postsurgical wound healing. Kramer observed a statistically greater amount of hydroxyproline production in "megadosed" guinea pigs than in control animals at 7 days after surgery.[15] Yew evaluated the time it took scabs of wounded guinea pigs to detach. It was determined that the number of days it took for the scab to detach was shorter in animals on high dosage.[68] Other investigators observed no enhanced wound healing using high dosages of vitamin C.[34,69]

Human wound studies using "megadoses" of vitamin C are inconclusive. Cheraskin observed improved gingival wound healing in dental students on high doses of vitamin C.[70] Woolfe evaluated healing of periodontal flap surgery using megadose levels in humans and observed no significant differences from controls who were on recommended daily allowance (RDA) levels of ascorbic acid.[71] Improved clinical healing has been

observed in the treatment of pressure sores and burns.[72] Megadose therapy was utilized in the treatment of immune disorders such as Chediak-Higashi syndrome and Hyperimmune E, as well as collagen disorders such as Type VI Ehlers-Danlos syndrome.[1,14]

Harmful effects from megadose therapy have been discussed in the literature. These include renal stone formation, gastrointestinal problems, megadose conditioning, vitamin B_{12} destruction and increased urinary histamine levels.[1,73] Most healthy adult humans and guinea pigs tolerate massive doses of ascorbic acid without significant effects.[74,75,76,77,78,79,80,81,82,83,84] Once tissue saturation has been achieved, excess amounts of vitamin C are released through the respiratory, urinary and fecal routes.[79,76,85,81] Megadoses are only partially absorbed when orally administered, due to the inability of the small intestine to efficiently absorb large doses and the relatively short period of time the ascorbic acid is retained in the gastrointestinal tract.[86] Multiple doses given in small amounts are readily absorbed.[23,19] Giroud observed that oral doses of greater than 50 mg. would not increase tissue saturation in guinea pigs.[87] Five hundredths of a milligram of ascorbic acid was

adequate to prevent pulpal degeneration in the incisors.[54,88,5] These dosages did not take into consideration individual needs and variability in guinea pigs.[89]

Healing wounds in guinea pigs have been evaluated by testing for physical strength, by histologic analysis and biochemical analysis. Collagen formation is an important constituent of each of the evaluations.[90,91] The variable is ascorbic acid supplementation.

Tensile strength studies evaluated the amount of force or air pressure needed to reopen a previously created wound. Results indicated a linear relationship between the dosage of ascorbic acid and the strength of the wound.[92,93,94,95,69] The optimum dosage was determined as the dosage above which wound strength did not significantly improve. Guinea pig wounds that were allowed to heal and then were depleted of ascorbic acid were found to have less tensile strength than control wounds, indicating the need for a continuous source of ascorbic acid to maintain tissue integrity.[96,97]

Histochemically, hydroxyproline production increased significantly in healing wounds of supplemented guinea pigs. Deficient guinea pig wounds had little, if any, collagen production.[68,15,52] Alkaline phosphatase also decreased in wounds of guinea pigs

depleted of ascorbic acid, while acid phosphatase increased.[55]

Histologic observations correlate well with tensile strength and histochemical evaluations. In skin and oral mucosal wounds, a fibrin clot forms, followed by a lag period of 2 to 3 days, during which time phagocytosis of damaged and necrotic tissue occurs. There is fibroblast and capillary infiltration into the fibrin clot to form granulation tissue following the lag period.[98,99] Epithelial migration begins at 3 to 6 days, moving at a rate of 0.5 mm./day over the granulation tissue and under the scab.[100] Collagen formation and maturation of tissue increases over time. By 1 week, epithelium covers all or part of the wound site.[91,101] As connective tissue matures, the underlying bone remodels and reforms. Size of the wound may or may not be a factor in the rate of wound healing. Spain observed larger tongue wounds healed at a faster rate than smaller wounds in the guinea pig.[102] Stahl compared healing of large and small oral wounds in the adult rat and observed a delayed healing of larger wounds due to the presence of debris and persistent inflammation.[103] Severity of the wound, infection and nutritional status of the individual all

affect the stage of wound healing.

In skin wounds of deficient guinea pigs, Hartzell observed delayed proliferation of the fibroblasts with little or no collagen production. Edema and bleeding into the wound site continued for an extended period of time. Overall healing was delayed for 1 week. Tensile strength never achieved the same level as in control animals.[92] Bourne and Hunt observed increased cellular proliferation, multiple effusions, immature intercellular substance and immature cells in healing wounds. Collagen degradation was evident.[91,99] Lanman observed minimal collagen production, persistent granulation, hemorrhaging and irregular fibroblast arrangement in wounds of marginally deficient guinea pigs.[49] Yew and Veen-Baigent showed extended scab adhesion in marginally deficient guinea pig wounds.[68,34] Veen-Baigent also observed significantly enlarged hearts, kidneys, adrenal glands and spleens in deficient animals.

Barr made standardized wounds in the femur and oral mucosa of deficient guinea pigs with a trephine bur. At 7 days, osteoid formation occurred in the femur control sites. The wounds of deficient animals lacked fibrous organization and contained heavy, amorphous, cellular infiltrate.[104] Oral wound sites of

deficient animals displayed inadequate collagen production and delayed wound healing.[105] Collins observed delayed trephine wound healing in the oral mucosa of the labial mandibular incisors.[106] Trephine burs provide a standard wound site to allow for uniform comparisons. Bassetti created oral wounds in rats to study the effects of chlorhexidine on wound healing. A quantitative analysis of histologic wound sections was made comparing tissue zones between groups.[107]

A histologic comparison of standardized wounds in guinea pigs receiving different levels of ascorbic acid was accomplished by modifying the technique of Bassetti. Determinations were made to distinguish if varying doses of vitamin C affected oral wound healing in guinea pigs. Body weights, plasma ascorbic levels and organ weights were also compared.

MATERIALS AND METHODS

Forty-five Murphy/Hartley guinea pigs[I] with an initial weight of 350 to 400 gms. were randomly placed into four groups. One animal died of a nonspecific cause prior to grouping. The groups consisted of 14,10, 10 and 10 animals. Each animal was tatooed on the left ear for identification: numbered 00 through 44. Two animals were housed in each cage. The animals were maintained at the Veterinary Sciences Division, Wright-Patterson Air Force Base. Animals were fed Purina Guinea Pig Chow (Appendix A)[II] for 70 days (Figure 1). Animals were placed on a vitamin C deficient pelleted diet (Appendix B)[III] for 14 days.[94,52,105,34,89,33,55]

Following ascorbic acid depletion, the animals were continued on the ascorbic acid deficient diet. The groups of animals were supplemented with varying levels of ascorbic acid. Vitamin C was dissolved in a 20% sucrose and water solution (Appendix C) and was orally administered with a 10 cc. syringe and plastic tube. Supplements were given each morning.

GROUP I 0.5 mg.: The minimum dosage of vitamin C required to prevent clinically overt

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I - Murphy Breeding Laboratories, Inc., Plainfield, Ind. 46168.
II - Ralston Purina Co., PO Box 548, Richmond, Ind. 47374.
III - ICN Nutritional Biochemicals, PO Box 28050, Cleveland, Ohio 44128.

signs of scurvy.[65,8,34,68,5,88]

GROUP II 5.0 mg.: Adequate dosage of vitamin C[65,15, 68,106,34,93,5]

GROUP III 50.0 mg.: Tissue saturation dosage of vitamin C[34,68,106,54,87,108]

GROUP IV 250.0 mg.: Megadose level of vitamin C[15,34, 68,76,79]

Supplements were administered for 28 days so that the animals would become conditioned to the amount of vitamin C.[9,65] The weights of the guinea pigs were determined and recorded twice weekly in the morning after administration of the supplement.

On day 28, all the animals were anesthetized with a combination of Ketamine[I] and Xylazine[II] (Appendix D).[109] The anesthetic mixture was given in a dosage of 0.5 cc./kg. body weight, intramuscularly, in the hind leg using a 1.0 cc tuberculin syringe. One group of animals at a time was anesthetized. Each animal was laid on its back on a towel-covered board and a rubber band was looped over the maxillary incisors. A modified rubber dam forcep was used to retract the buccal tissues. The

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I - Vetalar (Ketamine Hydrochloride), Parke-Davis, Div. of Warner-Lambert Co., Morris Plains, N.J. 07950.
II - Rompun (Xylazine), Haver-Lockhart, Bay Vet Division/ Cutter Laboratories, Inc., Shawnee, Kansas 66201.

tongue was retracted with a periosteal elevator and the mandibular incisors were pulled away for direct vision of the surgical site. The premaxilla was chosen as the site to create a standard wound. A trephine bur,[I] 2.3 mm. in diameter, was inserted in a variable speed handpiece to make a uniform wound in the midline of the oral mucosa, anterior to the maxillary premolars. (Figure 2). The trephine bur penetrated the tissues until it met resistence from the underlying bone. An eight-round carbide bur was used to penetrate the surface of the bone to mark the site. The wound was rinsed with saline and the debris was suctioned with a pipette attached to a faucet vacuum unit. The animals were wrapped in towels to maintain body temperature and were returned to their cages. Towels were removed following recovery from anesthesia.

Thirty-six days following the beginning of supplementation, each group of animals was anesthetized with Ketamine and Xylazine. Approximately 8 cc. of whole blood was removed via heart puncture with a 20 cc. disposable syringe and 18 gauge needle. The blood sample was injected into two foil-covered blood collection tubes. The blood samples were stored in a refrigerator

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I - 150-023 Trephine Bur, Phingst Busch Co., Germany.

at 5°C for 1 half hour. The blood was centrifuged for 5 minutes and plasma was extracted. The combined plasma was then injected into a third collection tube and stored in the refrigerator. All samples were collected at the end of the day and placed in ice.

Plasma samples were analyzed for ascorbic acid by the Department of Toxicology, Hamilton Hall, The Ohio State University (Appendix E). The plasma was frozen at -5°C and stored for 1 week. Samples were thawed and metaphosphoric acid was added to prevent spontaneous ascorbic acid oxidation. Control samples of known amounts of ascorbic acid were plotted and graphed using high performance, liquid, chromatographic (HPLC) analysis. Linear regression was used to determine the best fit line, using values of 0.1 ug./ml. to 40.0 ug./ml. The plasma samples were analyzed and the values determined by comparing the peak heights to the standard curve. The HPLC analysis is sensitive at concentrations of 0.1 ug./ml. and total recovery approaches 100%. Plasma ascorbic acid concentrations were recorded and compared.

Immediately following blood collection, the animals were given a 3 ml. lethal dose of sodium pentobarbital[I]

I - Uthol Solution, 5 gm./ml., 100 ml. vial, Butler Co., Columbus, Ohio 43228.

by intracardial injection. An abdominal incision was made and the kidneys, adrenal glands and spleen were removed. The kidneys and adrenal glands were weighed in pairs on an Ainsworth analytical scale. The weights were recorded.

The maxillary jaw was removed with a Stryker saw and Ochsenbein chisels. A block section of the wound site was removed. The first premolars were retained within the block to aid in proper orientation of the maxilla in paraplast (Figure 3). The biopsies were fixed in 10% neutral buffered formalin for 4 days, photographed and washed in tap water. They were placed in individually labelled gauze sacks and decalcified in a solution containing formic acid and sodium citrate.[I] Radiographs were taken at 7 and 14 days and were used to determine that decalcification was complete at 2 weeks, except for portions of the premolars. Calcified areas were removed. The tooth side of the biopsy was labelled with India ink. The specimens were placed in metal cassettes, washed for 24 hours and processed on the autotechnicon. The sequence and time of solutions were: 70% alcohol, 1 hour; 80% alcohol, 1 hour; 95%

I - Formic Acid- Sodium Citrate Decalcifying Solution (one part 50% formic acid- one part 20% sodium citrate, water), The Ohio State University, Columbus, Ohio 43210.

alcohol, ½ hour; 100% alcohol, 2 hours; 100% alcohol, 2 hours; xylene, 1 hour; xylene, 2 hours; paraplast, 1 hour; paraplast. Specimens were retained in paraplast until imbedded. The infiltrated specimens were oriented in plastic wells so that the side labelled with India ink was facing the bottom of the well. The masticatory mucosa was oriented perpendicular to the base of the well.

Cross sections of the wound site were cut on a Universal Microtone. Serial sections were made at a thickness of 6 microns and the twentieth and twenty-first sections were mounted on separate slides. The specimens were fixed on a warming tray. They were deparaffinized and rehydrated through the following solution series: two changes of xylene, two changes of absolute alcohol, one change of 95% alcohol and distilled water. Alternating slides were stained with hematoxylin and eosin and Masson's stain for histologic evaluation. The specimens were then mounted in Canada balsam under cover slips.

The sections were examined using a Zeiss binocular microscope. The widest expanse of the healing wound was determined by using a 7 mm. square quadratic test grid in the viewing area at a magnification of 31X. A representative slide stained with hematoxylin and eosin

and Masson's stain was selected for histologic and histomorphometric evaluation. Slides were randomly numbered and evaluated impartially. The following tissue zones were evaluated:

1. Zone of Epithelialization: identifying criteria included cellular proliferation, flattening and elongation of epithelial elements at the wound margins, keratin formation, rete peg formation and widened germinatival layer with many mitotic figures.
2. Zone of Fibrin Clot Formation: identifying criteria included a dense inflammatory cell infiltrate entangled in a amorphous fibrin network.
3. Zone of Granulation Tissue: identifying criteria included cellular proliferation, vascular proliferation with capillary invasion, deposition of intercellular substances and fiber formation.
4. Zone of Residual Mature Connective Tissue: identifying criteria included decreased cellularity, fibrous proliferation, mature, thickened collagen fibers, presence of osteoclastic activity and presence of osteoblastic activity.

These zones were evaluated within an area encompassing the grid overlaying the central part of the wound. The center was determined by counting the

number of grids across the wound site and dividing by two. This area encompassed the edges of the bone wound or residual mature soft connective tissue. The center grid was placed over an identifiable landmark in the middle of the wound. The top row of grids o rlayed the surface of the wound. All grids that contained greater than 50% soft tissue were counted. Bone, teeth, artifactual spaces and cystic tissue were not included in the counts, but were described histologically. Tissue zones were measured by counting the number of grids containing each type of tissue in the wound area (Figure 4). Four randomly selected slides were evaluated by the chief investigator and another evaluator to determine accuracy of counting the wound sites. The number of grids counted in each zone were expressed in percentages of the wound area covered by the grid.

The following dependent variables were analyzed and compared for each group using a one way analysis of variance (ANOVA)[112]: the ratios of organ weights divided by body weights (kidneys, spleen and adrenal glands); the average body weight change from day 0 to day 28 and from day 28 to day 35; and the percentages of tissue zones (epithelium, fibrin clot, granulation tissue and residual mature connective tissue). Where

the ANOVA was significant, a *posteriori* pair-wise comparisons were done using Duncan's New Multiple Range Test.[113]

RESULTS

The ascorbic acid free diet was well tolerated by the guinea pigs. Supplementing the guinea pigs was difficult, for groups receiving 50.0 and 250.0 mg. of ascorbic acid, due to the acidity of the solution. Allowing the guinea pig to chew on the plastic syringe tubing and administering the supplement slowly aided in administering the proper dosage. Five animals died from pharyngeal or esophageal abscesses prior to wound formation. One animal died in Group III and two animals each died in Groups I and IV. No animals died following surgical wounding.

From day 0 to day 28, all groups gained weight, but this weight gain was not significantly different ($F=.49$, $df=3/35$, $P>.50$). Significant weight differences following surgery were observed from day 28 to day 35 ($F=5.26$, $df=3/35$, $P<.005$). Guinea pigs receiving 0.5 and 5.0 mgs. of ascorbic acid each day lost weight, while those receiving 50.0 and 250.0 mg. of ascorbic acid each day gained weight. Statistically significant weight differences ($P<.05$) occurred between Groups I and III, I and IV and between Groups II and III.

Guinea pig organ weights were determined at sacrifice. The ratios of the organ weights to the body weights were calculated and compared (Appendix H).

Kidney ($F=2.83$, $df=3/35$, $P=.0514$) and spleen ($F=.814$, $df=3/35$, $P>.50$) to body weight ratios were not significantly different between groups. Kidney to body weight ratios, however, were increased in Groups I and II. These values approached a significant level. Adrenal gland to body weight ratios were found to be significantly different ($F=3.03$, $df=3/35$, $P=.0514$). Groups III and IV were significantly higher ($P<.05$) when compared to Group I.

Ascorbic acid plasma levels were not detectable in Groups I and IV. Minimal levels of ascorbic acid were observed in four samples from Group II and three samples from Group III (Appendix I).

Clinically, the oral wounds appeared uniform in size after 8 days. All wound sites were covered by soft tissue. Wound shapes varied from ovoid to circular (Figure III). Wound sites were visually detectable and block sections were removed easily.

A histologic section was taken from an animal not surgically wounded (Figure IX). The epithelium was orthokeratinized, 50 to 60 cells thick and rete peg formation was evident. The underlying connective tissue was fibrous and cellular. The tissue was vascular; containing arterioles, venules and capillaries. The connective tissue adjacent to the underlying bone contained thickened collagen fibers.

Osteoblastic activity produced an atypical bony pattern (chondroid bone).[7] Mature osseous tissue was unremarkable. The overall bony pattern was trabecular with highly cellular connective tissue interspersed between the trabeculae. The roots of the teeth were observed beneath the bone. The thickness of the premaxilla site was 1.5 to 2.0 mm.

The surgical wounds varied in size, ranging in width from 2.5 to 4.0 mm. between the bony margins. The widest wound sections were used to evaluate the central wound site. Each tissue zone was counted with the grid and the ratio of tissue type to the total tissue area was calculated (Appendix J). Statistically, there were no differences ($P>.05$) between the tissue types (Table III).

The epithelium on the wounds covered all but five sites. In areas where it failed to cover, the marginal cells were flattened and elongated as they migrated over the granulation tissue. Exfoliating bone spicules were associated with these ulcerated sites. Cyst formation was observed in five wounds. Four of the cysts appeared to contain keratin. The fifth appeared to be odontogenic, consisting of ameloblastic cells lining the walls and producing an enamel matrix. Several wound sites exhibited epithelial downgrowth into the

granulation tissue or adjacent to the tooth surface.

The epithelium in the majority of the wound sites was keratinized, 10 to 50 cells thick and exhibited rete peg formation. The germinatival layer was widened and all epithelial layers were discernable.

Fibrin clot was characterized by an amorphous cellular zone with a dense inflammatory cell infiltrate. It was observed in 11 out of 39 specimens, eight of which were seen in Groups I and II (Appendix J). It was observed in the superficial area of the wound and occasionally encased in epithelium or associated with bone spicules. Fibrin encompassed over 50% of the area in three wound sites evaluated under the grid.

The zone of granulation tissue was distinguishable by the proliferating fibroblasts and endothelial cells. Vascular proliferation was determined by the numerous budding capillaries. Fiber formation was observed by the light blue staining of the immature collagen. Granulation tissue was covered by epithelium or fibrin clot in the central portion of the wounds. It was superficial to the underlying bone and blended into the residual mature connective tissue. Occasional inflammatory cells were observed within the granulation tissue. Vascularity and cellularity were uniform in all sections observed. Collagen fibers increased in

number and thickness towards the periphery of the wound sites.

Residual mature connective tissue was differentiated from granulation tissue by the dark blue staining of the mature collagen fibers. Other features including decreased cellularity of the tissue and an increase in intercellular substance were located at the periphery of the wound site below mature epithelium. Three histologic sections did not contain mature connective tissue within the grid area. These were the same wound sites that contained over 50% fibrin.

Osseous tissue was observed in a state of simultaneous osteoblastic and osteoclastic activity. Osteoclasts lined the immediate periphery of the bone margins, as well as, the opposing side of the trabecular bone. Exfoliating bony spicules were occasionally located in the granulation, epithelial and fibrinous tissues. This bone was nonvital and appeared to impede the healing of adjacent tissues. Active atypical bone formation was observed in all but three histologic sections. This chondroid-like tissue was highly cellular and stained a lighter blue when compared to mature bone tissue. Bone formation was observed along the peripheral borders of the wound, as well as, in sites

devoid of adjacent bone. These areas were probably cross sections of new trabecular bone formation.

Bone was not observed in several sections covering the roots. Occasionally, roots appeared damaged from the bur during surgery. In other areas, roots were undamaged and bone was not evident due to extensive bone resorption. Histologically, ascorbic acid had no effect on morphologic bone formation or resorption in any group of animals.

DISCUSSION

The effects on wound healing by varying levels of ascorbic acid were evaluated in guinea pig oral mucosa. Four specific dosages of ascorbic acid were given. Group I was given 0.5 mg. of ascorbic acid. This supplement was determined to be the minimal amount of vitamin C needed to prevent clinical signs of scurvy in guinea pigs.[65] Group II received 5.0 mg. each day, which was considered an adequate amount to maintain health and prevent histopathologic signs of scurvy.[88] Group III received 50 mg. of ascorbic acid each day. This amount is generally considered to be enough to obtain tissue saturation in the guinea pig.[34] Group IV was given 250 mg. of vitamin C each day, which was designed to determine if massive doses would effect the healing of oral wounds.[15]

In this study oral wound healing in guinea pigs was not affected by varying the level of ascorbic acid. A modification of the technique by Bassetti was used to quantify wound healing at 8 days. Bassetti observed that topical application of chlorhexidine disturbed the progression of wound healing. Specimens showed: a reduced or absent epithelial surface, retention of fibrin clot and granulation tissue and lack of mature connective tissue. Statistically, oral wound healing in rats was inhibited by high levels

of chlorhexidine.[107] There was no statistical evidence of delayed or enhanced wound healing in the present study. There were variations in tissue zone to total tissue area ratios within each group. Other factors affected the healing of individual wounds. Exfoliating bone, cyst formation, variation in the size of the wound and food retention were observed and could have altered the progression of healing in the animals.

Varying the ascorbic acid levels did not affect epithelial migration and maturation in the wounds. Investigators have produced varying effects after depletion of ascorbic acid. Johansen observed epithelialization and keratinization in 5 days.[101] Hunt observed no effects of partial ascorbic acid depletion on epithelial formation in abdominal wounds of guinea pigs.[99] Collins, however, observed enhanced epithelial coverage in oral wounds of guinea pigs.[106]

Fibrin clot was observed in eight out of 22 specimens in groups supplemented with 0.5 and 5.0 mg. of ascorbic acid. Three out of 17 sections in groups supplemented with 50 and 250 mg. contained areas of fibrin at 8 days. Delayed wound healing would be expected to show that the fibrin clot with inflammatory cell infiltrate would be retained. Hunt observed

retention of amorphous fibrin in abdominal wounds of partially deficient guinea pigs.[99] Bone wounds in acutely deficient guinea pigs showed a continued presence of fibrin for 7 days within the wound site.[104] Perhaps evaluating oral wounds at 8 days allows healing to progress to such an extent that differences in the rate of healing can no longer be detected. In the present study, ascorbic acid had no effect on the formation or retention of the fibrin clot. Prior to this study, wound healing was observed histologically at 5, 7, 12 and 17 days. Eight days was chosen due to the fact that epithelialization was completed in the wounds of the pilot study.

Granulation tissue was determined histologically by the presence of proliferating fibroblasts and budding capillaries lined by endothelial cells. Early collagen formation and organization was observed at 8 days. These findings correspond to those found in animals adequately supplemented with ascorbic acid. Granulation tissue developed and matured regardless of the level of ascorbic acid supplement. Turesky observed a decrease in vascularization and collagen organization, and a lack of maturation of granulation tissue in acutely deficient animals after 10 days.[52] Hunt reported continued stimulation of fibroblast

proliferation, reduced capillary invasion and retained immature connective tissue in partially deficient animals.[99] In animals supplemented with high doses of ascorbic acid, Kramer determined a significant increase in hydroxyproline production in 7 day wound sites.[15]

Residual mature connective tissue included mature collagen fibers, intercellular substance and a decrease in cellularity. In this study, high levels of ascorbic acid did not enhance connective tissue maturation and low levels did not adversely inhibit it. Evaluation of wound healing at 8 days in guinea pigs may have been too early to determine formation of mature connective tissue.[99] Other researchers observed a delayed maturation of collagen in wounds of acute and partially depleted animals.[10,51,105] However, Claycomb reported that oral collagen in guinea pigs maintained a high, continuous level of formation. He observed this in ascorbic acid deficient animals. Collagen metabolism was also greater in the oral mucosa than in the skin of guinea pigs.[48] His results support the findings in this paper, which indicate that collagen maturation was not significantly different in animals receiving various levels of ascorbic acid.

Osseous tissue was not one of the dependent

variables evaluated quantitatively in this study. Bone spicules near the wound surface were associated with ulceration of the wound. The bony periphery of the wound was lined with osteoclasts and osteoblasts, indicating active bone remodeling. Bone formation has been found to be delayed in acute and marginally deficient animals.[56,104] Bourne determined that ascorbic acid deficient animals formed a reduced number of trabeculae. Hunt assessed bone activity in deficient guinea pigs and concluded that, except for a slight increase in osteoclastic activity, there were few differences in adequately supplemented animals.[58] The findings in the present study tend to agree. Various levels of ascorbic acid had no obvious effects on bone remodeling.

Bone formation was unusual around the molars of the guinea pig. The highly cellular immature tissue was cartilagenous in nature and is called chondroid bone.[7] Almost all the sections observed contained chondroid tissue. As the tissue matures, it becomes less cellular, calcifies and takes on a bony appearance. Chondroid tissue warrants further study, using the guinea pig as the animal model.

In this study, wound healing was not always consistent. In some wounds the bur had penetrated

through the bone. The underlying teeth were occasionally damaged and may have affected the healing progression. The use of the second bur may have caused this variation and was probably unnecessary following wounding with the trephine bur. Bone spicules were sometimes found in the wound site even though the wounds had been irrigated and evacuated carefully. Wound widths were not always uniform. This could have been due to excessive lateral movement of the bur, increased osteoclastic activity prior to sacrifice or processing of the specimens.

It is of interest that an odontogenic cyst was observed in one of the specimens. An excessively deep wound allowed ameloblastic cells to migrate toward the surface from the continuously erupting incisor. Matrix formation was observed within the cyst, indicating the specialization of the cells. Keratin formation was observed in four other cysts seen in the specimens.

Four randomly selected slides were counted by two examiners to determine accuracy. Sixteen tissue zones were counted and the examiners were found to be accurate within two grids in each zone. All sections were randomly counted at least three times and the number of grids from each zone was averaged to reduce the chance for error. Counting errors could have occurred due to improper centering of the grid,

tangential sectioning of the blocks or distortions of the specimens during processing of the tissues.

Varying the levels of ascorbic acid had no effect on the overall healing of oral wounds in the guinea pigs at 8 days. The method used may not have been sensitive enough to detect differences between the tissue zones of each group. Various investigators have determined that ascorbic acid is mobilized and concentrates in areas of healing and high metabolic activity in the body. This occurred in the guinea pig, regardless of the amount of vitamin C given to the animal.[37,38,39,94] This could explain why there were no differences in wound healing. All wound sites may have received enough ascorbic acid to permit adequate healing. Schwartz maintains that even minimal amounts of ascorbic acid are sufficient for wound healing.[91] Wilson observed that vitamin C was absorbed through the buccal mucosa.[115] This should be considered in this study, since the animals received orally administered ascorbic acid.

It is difficult to make direct comparisons of this study to others, due to differences in methods. Studies that compared tensile strength of wounds observed differences in acutely and partially deficient animals.[92,93,94] Yew and Veen-Baigent used a gradient

of ascorbic acid levels to determine the effect on scab adhesion in guinea pigs. Although they observed extended scab adhesion in minimally supplemented animals, increased levels of ascorbic acid had no significant effect on reducing the time scabs were retained. Hydroxyproline and hydroxylysine production increased linearly with higher supplements of ascorbic acid. There were no increases in these collagen components when animals received high supplements of vitamin C.[34,68] However, Kramer observed increased hydroxyproline production in skin wounds of highly supplemented animals at 7 days. The apparent discrepancy can be explained by the timing of the evaluations; Veen-Baigent evaluated the wounds at 8 weeks and Kramer made his determinations at 7 and 14 days. In the present study, comparing wound healing at various time intervals after surgery may have demonstrated significant histologic differences.

Clinically, all animals received ascorbic acid supplementation via oral administration. Group II received daily amounts of vitamin C considered to be adequate for normal health and well being.[7,15,54,106] A designated control group receiving adequate amounts of ascorbic acid in food was not considered appropriate. The uptake of vitamin C by the animals would have been difficult to determine.

Plasma levels of ascorbic acid were determined in this study to calculate if there was a linear relationship between supplement levels and the amount found in the plasma. Only seven animals from Groups II and III had detectable levels of ascorbic acid in the plasma. There are several possible explanations for these results. Blood samples were removed 24 to 32 hours after final supplementation. Ascorbic acid is not stored in the plasma. It is transported in the blood to various sites in the body, where it is stored and utilized.[32,40,85] Therefore, minimal or no ascorbic acid would be found in the plasma. However, studies have indicated the presence of vitamin C in acutely deficient animals.[35,80,92,99] Plasma levels are not considered to be completely reliable and only indicate metabolic turnover rate.[29,35] It may fluctuate during the day.[30] All of the animals in this study were placed on a 2 week ascorbic acid deficient diet in order to reduce tissue stores. They were given oral supplements of ascorbic acid, which stressed the animals. Groups III and IV were stressed more, due to the extreme sour taste of the solution. It is interesting to note that investigators have not indicated problems administering high levels of ascorbic acid orally to guinea pigs. In this study, oral

administration of ascorbic acid was difficult and stressful. Surgery was performed on all animals. The combination of all these events contributed to the reduction of ascorbic acid in the animals. Stress from surgery has been shown to reduce plasma levels of ascorbic acid.[1,68] Inducing a scorbutic state in animals decreases their ability to absorb ascorbic acid, especially high doses.[5,19,23,26,97] Various anesthetics have been shown to lower levels of ascorbic acid.[28,116] In summary, the lack of plasma ascorbic acid may have been due to stressful procedures, poor absorption, anesthesia or obtaining blood samples after the vitamin C was utilized and stored in the tissues. Evaluation of ascorbic acid levels in tissues of the adrenal gland would probably have been a better approach in determining individual animal stores.[5,34,65]

A standard approach in determining adequate supplements of ascorbic acid was to measure weight changes or growth in guinea pigs.[33,65] All animals were weighed twice a week from day 0 to day 28. The weight differences between groups were compared and found to be nonsignificant. All four groups gained similar amounts of weight. Group I, even though it received the minimal supplement of ascorbic acid, gained weight consistent with the other groups. This

study confirms the findings of others who gave guinea pigs 0.5 mg. of ascorbic acid each day.[8,58,65] However, individual variation in weight gains within each group was a common occurence. Williams and Deason observed a 20 fold weight variation in guinea pigs receiving various low levels of ascorbic acid. They noted a trend toward improved weight gain with higher supplements of vitamin C.[89] Other researchers have observed similar findings, with no increase in weight gain at high doses.[26,34,68,87] It appears that very low vitamin C supplements are a factor in reduced weight gain and well being of the guinea pig. In this study, various levels of ascorbic acid had no effect on growth of the guinea pigs.

After surgery, Groups I and II had a mean loss in weight. Groups III and IV showed a net gain in weight. Significant differences in weight were observed between Group I and Groups III and IV, as well as between Group II and Group III. One would expect a loss in weight following oral surgery, due to the discomfort from the wound. Animals given high levels of ascorbic acid recovered better than those on low doses. Yew observed similar results following surgery.[68] Ginter reported that guinea pigs given high doses of ascorbic acid had a delayed loss of weight when placed on a scorbutic diet.[78] In this study, increased levels of ascorbic acid

significantly enhanced weight recovery following surgical trauma. Systemically, high doses appeared to be of some benefit post surgically, but not to the observed injured tissues. This is an important consideration which should be investigated further.

Organs were weighed at sacrifice and the ratio of the organ weight to the overall weight of the animal was determined. Spleen to body weight ratios were not significantly affected by ascorbic acid supplementation. Ginter and Veen-Baigent observed enlarged spleens in chronically deficient animals after 68 days and 42 days, respectively.[34,67] In the present study, 35 days may have been too early to determine significant differences in spleen to body weight ratios.

Differences in kidney to body weight ratios approached significance (P=.0514). Other studies have observed kidney to body weight ratio increases after 5 to 10 weeks on marginal supplements of ascorbic acid. The organ weight gain was thought to be due to increased fluid retention.[34,65,67] Groups I and II in this study received vitamin C supplements that caused an increase in the kidney weights of these animals in relation to their total body weight.

Adrenal gland to body weight ratio values were significantly higher in Groups III and IV. These

results were in contrast to previous studies. Howard and Veen-Baigent observed a significant increase of organ size in ascorbic acid deficient animals, not in highly supplemented guinea pigs.[34,66] The discrepancy may have been due to the significant weight losses by the animals. The adrenal gland weights increased slightly and the overall body weights decreased significantly. This produced a doubling of the adrenal gland to body weight ratio in Howard's study. The adrenal gland enlargement was due to an increase in cell number and size, an increase in vascularity and an increase in the size of the medulla. In the present study, high levels of ascorbic acid supplementation produced more stress in Groups III and IV. This may have caused an increase in the size of the adrenal glands. Administering lower doses to Groups I and II appeared to have been less stressful. Also, the animals used in this study were initially much larger. The average weight at sacrifice was over 800 gm. Most studies have used animals that weighed in the range of 400 to 500 gm. Size and age of the guinea pig may have been a factor in all the dependent variables examined in this study and warrants further investigation.

Large single doses of vitamin C were not

efficiently absorbed by the guinea pigs. Other studies have shown smaller multiple doses of ascorbic acid were absorbed better than a single dose. Adequate levels were absorbed by the guinea pig and the remainder of the vitamin C was excreted in the urine.[79] The effects of continuous multiple supplements versus single daily supplements of vitamin C on wound healing warrants further study.

The study failed to show any benefit of massive doses of ascorbic acid in oral wound healing. This agrees with a recent human study by Woolfe.[71] Periodontal surgery was performed on patients supplemented with 1 gm. of ascorbic acid each day. Healing was not significantly improved. Cheraskin observed improved gingival wound healing in dental students administered 1 gm. of vitamin C each day.[70] The controversial role of nutrient supplementation will continue until improved methods of evaluating wound healing are developed.

CONCLUSIONS

1. Varying the level of ascorbic acid supplementation had no significant effect on oral wound healing in guinea pigs at 8 days.

2. Plasma ascorbic acid levels were detectable in only seven animals, regardless of the level of supplementation.

3. Varying the level of ascorbic acid supplementation had no significant effect on growth or weight gain from day 0 to day 28.

4. Varying the level of ascorbic acid supplementation significantly affected the weight of the guinea pigs post surgically, between the ascorbic acid deficient Group I and the high ascorbic acid supplemented Groups III and IV, as well as between the normal ascorbic acid supplemented Group II and the moderately high supplemented Group III.

5. Varying the level of ascorbic acid supplementation had no significant effect on the organ to body weight ratios of the spleen.

6. Minimal levels of ascorbic acid supplementation tended to increase the organ to body weight ratios of the kidney.

7. Increased levels of ascorbic acid supplementation significantly increased the adrenal gland to body weight ratios between the ascorbic acid deficient Group I and the high ascorbic acid supplemented Groups III and IV.

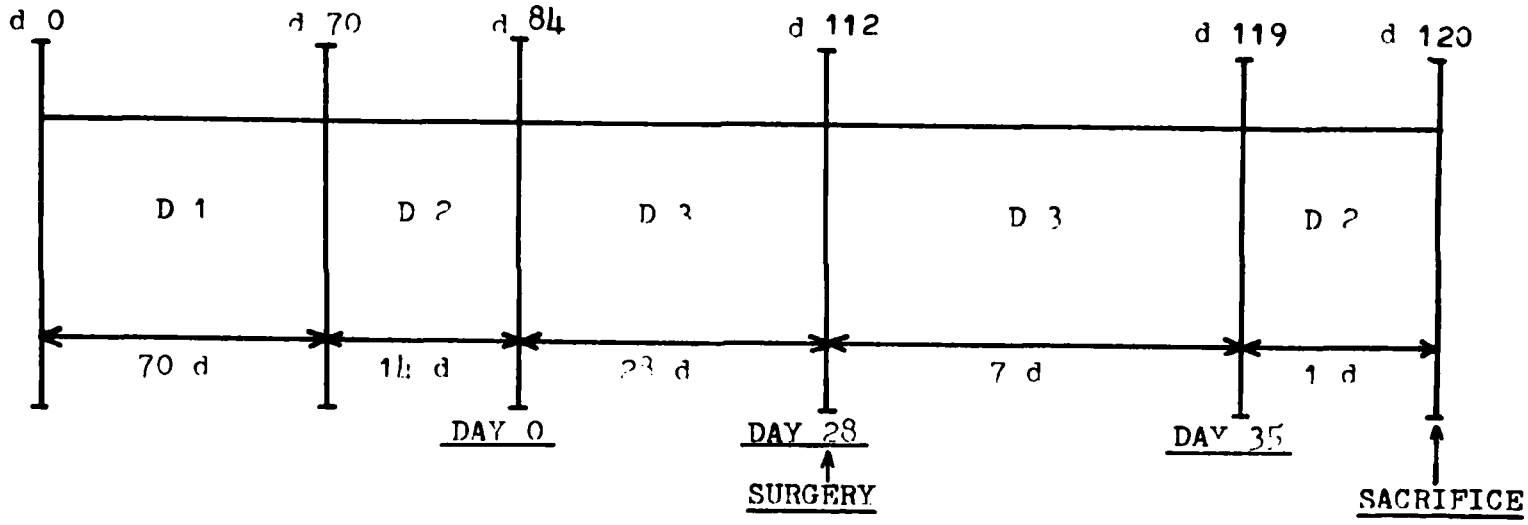
SUMMARY

Forty-five Murphy/Hartley guinea pigs were randomly placed into four groups and fed an ascorbic acid deficient diet for 2 weeks. Each group of animals then received a daily oral supplement of the following doses of ascorbic acid: 0.5 mg., 5.0 mg., 50 mg., 250 mg. All animals were weighed twice a week. Twenty-eight days later, a standardized wound was made in the premaxilla. On day 36, all animals were sacrificed. Blood samples were evaluated for levels of ascorbic acid. Thirty nine samples showed insignificant levels of vitamin C, 32 of which showed no ascorbic acid. Block sections of the premaxillary wound site were processed for histologic evaluation. Varying the levels of ascorbic acid had no significant effect on wound healing. Spleen to body weight ratios were not significantly affected. Minimal levels of ascorbic acid tended to increase kidney to body weight ratios and high doses caused a significant increase in adrenal gland to body weight ratios. Varying levels of ascorbic acid supplementation had no affect on growth prior to surgical wounding. However, increased levels of ascorbic acid enhanced weight recovery post surgically in the animals.

FIGURES

FIGURE I

SCHEMATIC OF EVENTS



D 1. REGULAR DIET (PURINA GUINEA PIG CHOW)

D 2. DEPLETION DIET (VITAMIN C DEFICIENT DIET)

D 3. DEPLETION DIET PLUS SUPPLEMENTS (VITAMIN C DEFICIENT PLUS SUPPLEMENTS)

FIGURE II
PREMAXILLA SITE OF WOUND

FIGURE III
BLOCK SECTION OF WOUND SITE

FIGURE IV
HISTOLOGIC SECTION OF WOUND AREA
GRID OVERLAY

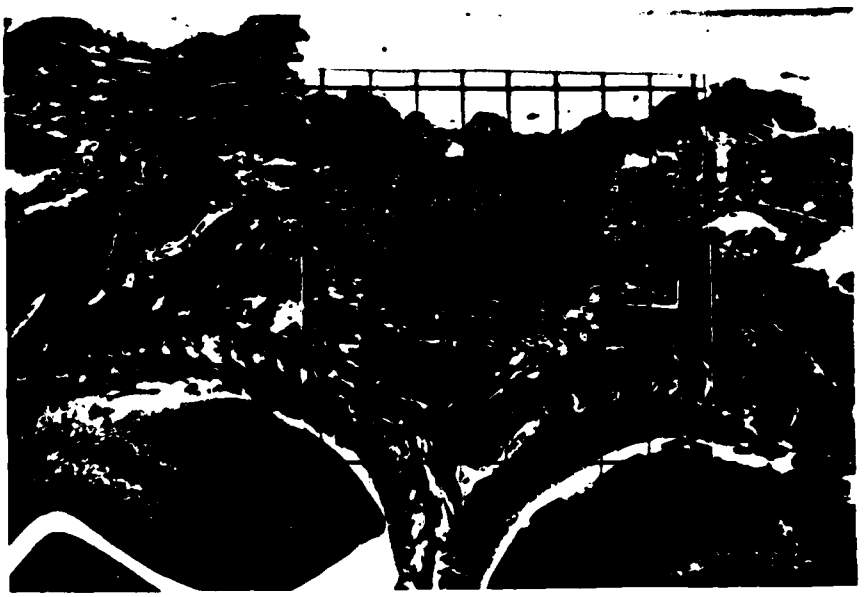


FIGURE V

DIFFERENCES IN BODY WEIGHT

DAY 0 VS DAY 28 (GM.)

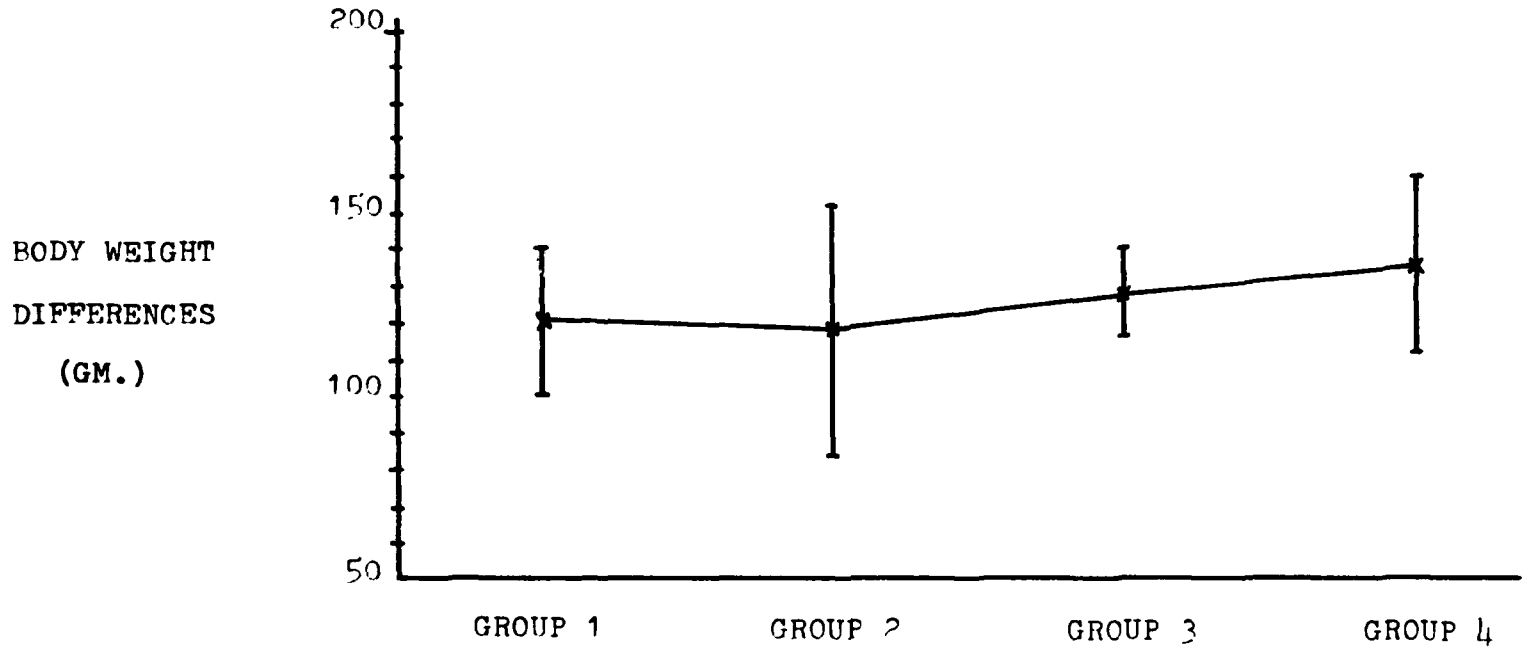


FIGURE VI

DIFFERENCES IN BODY WEIGHT (GM.)

DAY 28 VS DAY 35

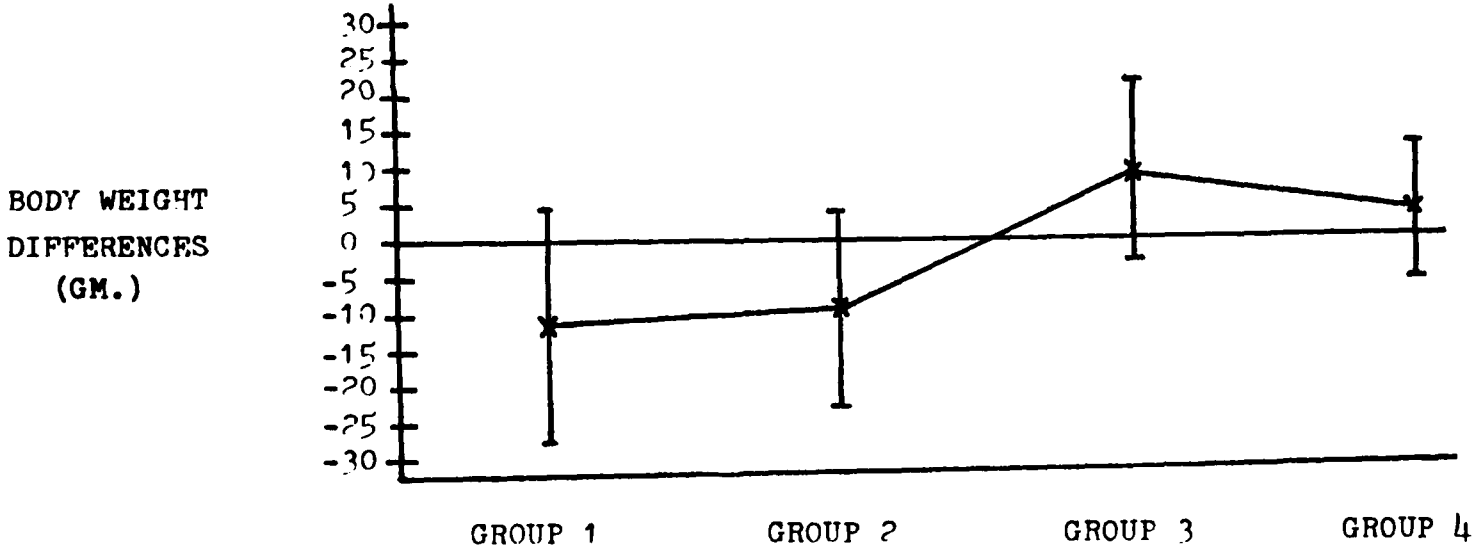


FIGURE VII

COMPARISONS OF ORGAN TO BODY WEIGHT RATIOS

KIDNEYS AND SPLEEN

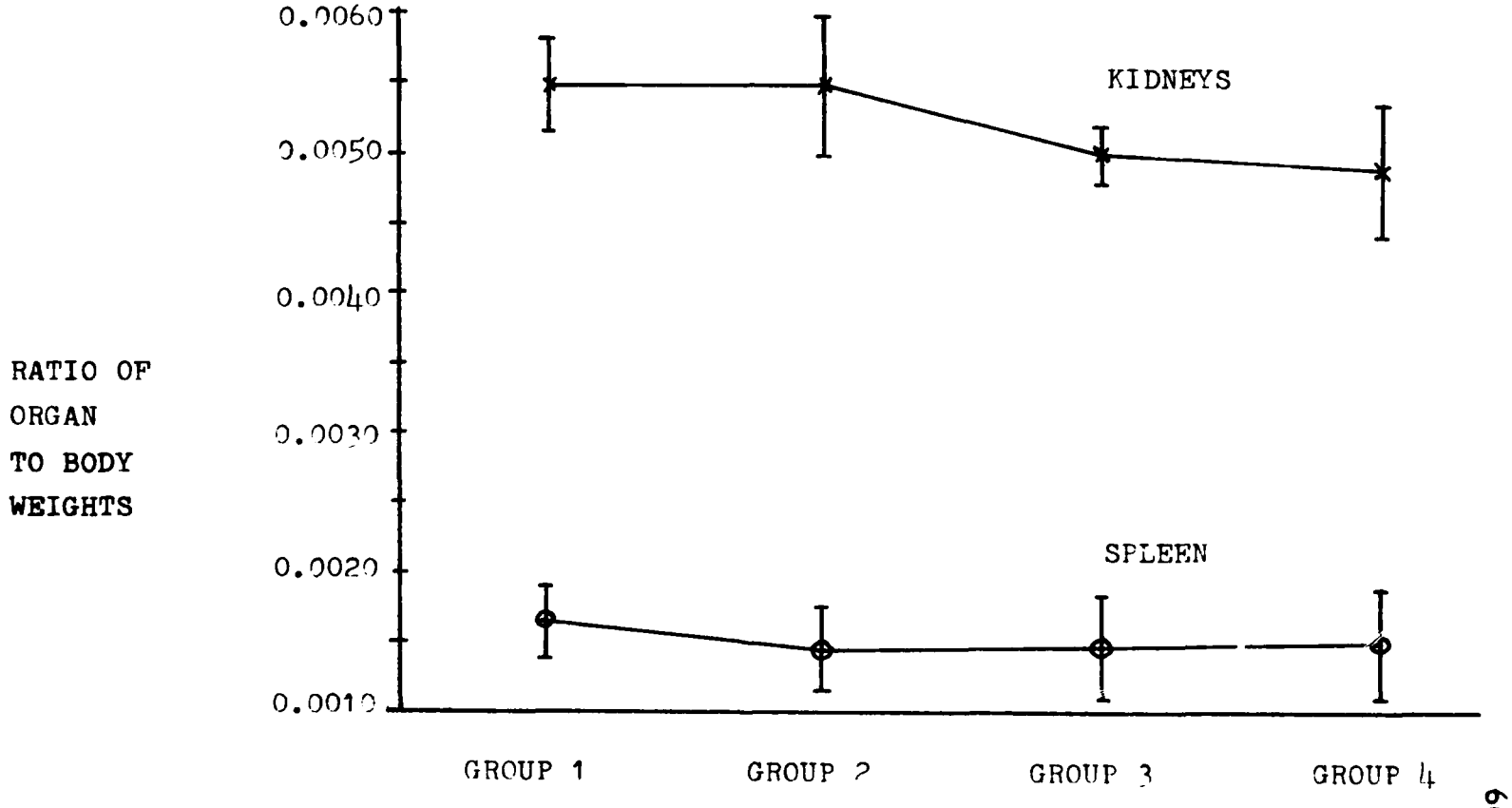


FIGURE VIII

COMPARISON OF ORGAN TO BODY WEIGHT RATIOS

ADRENAL GLANDS

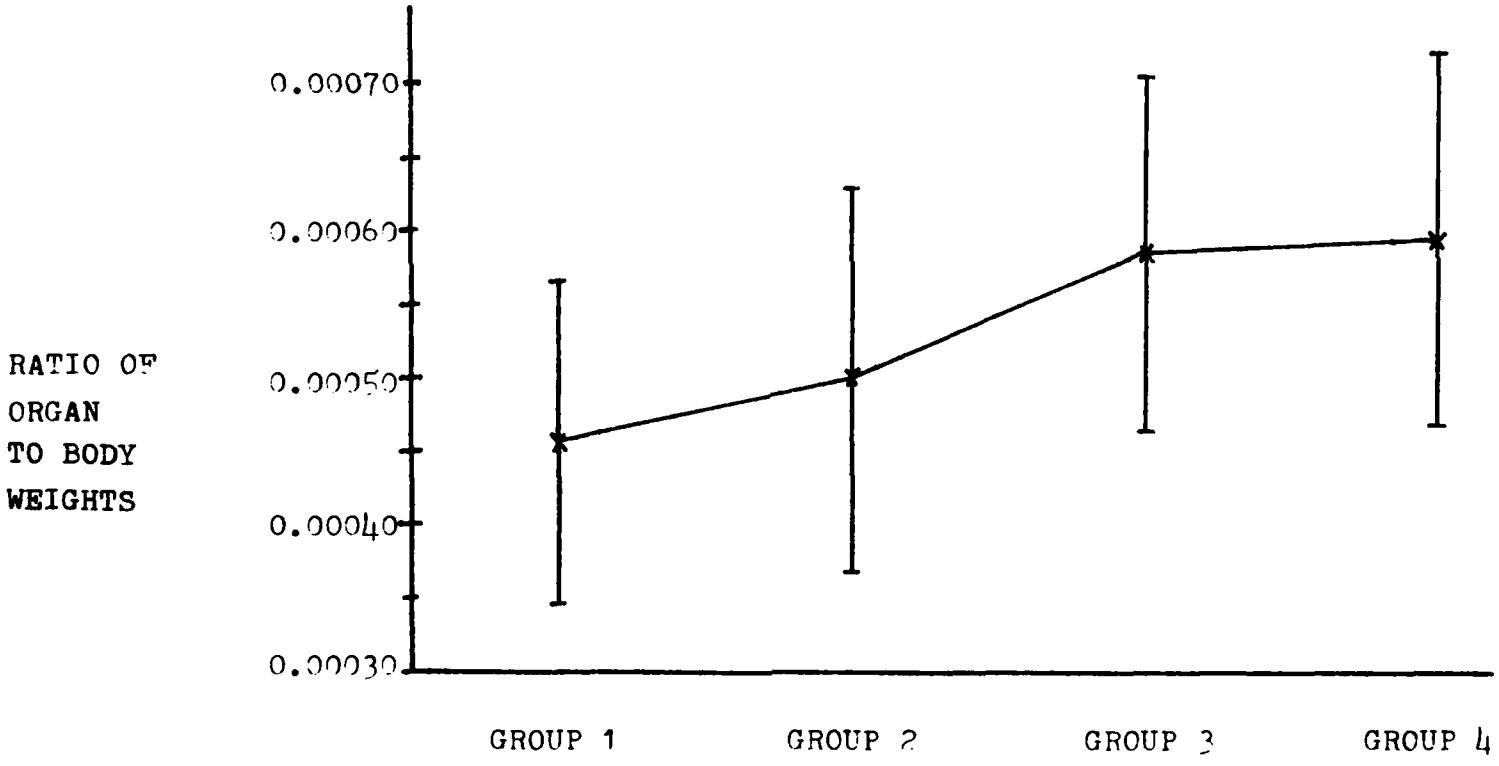


FIGURE IX
HISTOLOGIC SECTION OF PREMAXILLA

FIGURE X

COMPARISON OF TISSUE ZONES

EPITHELIUM

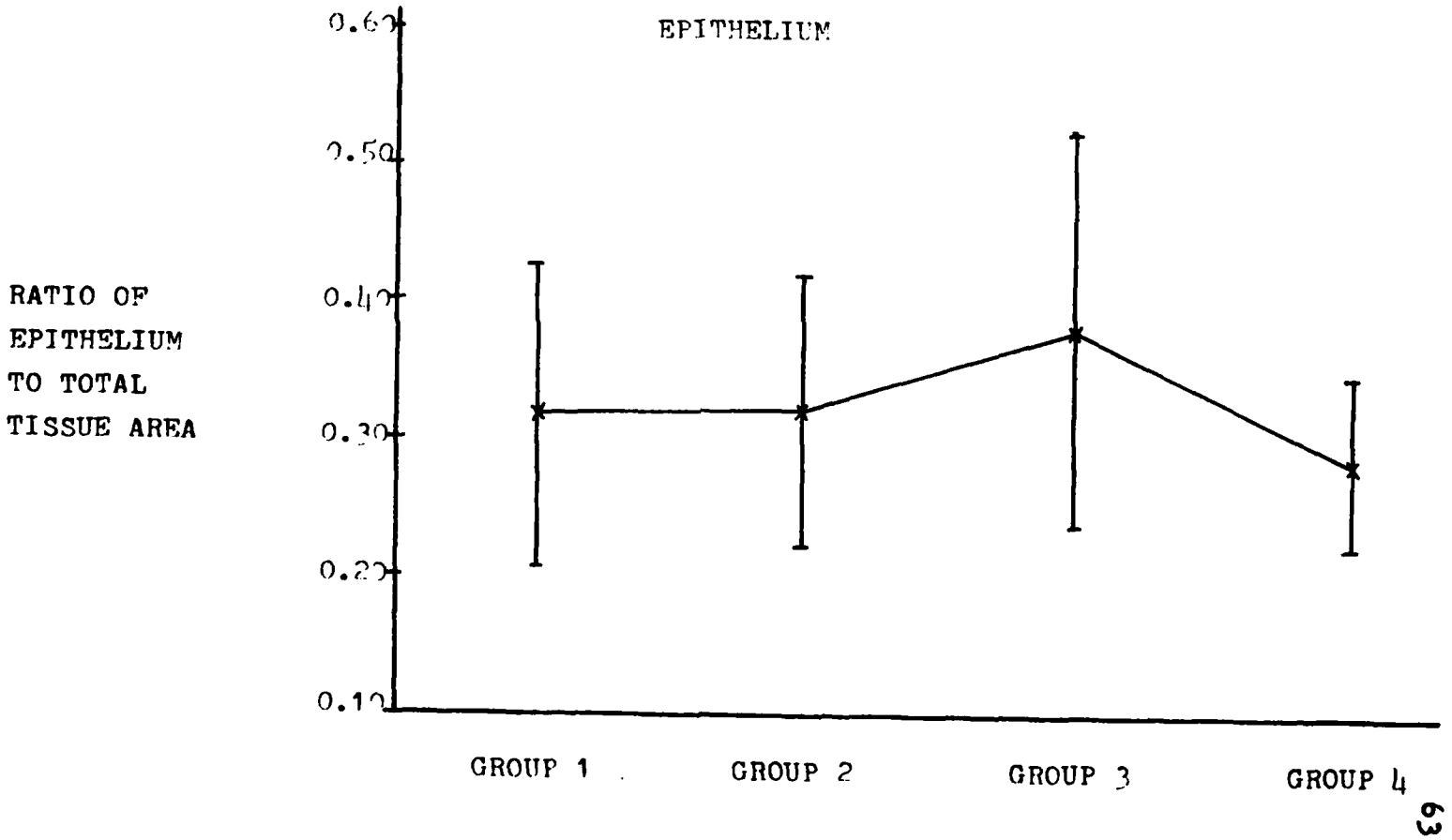


FIGURE XI

COMPARISON OF TISSUE ZONES

FIBRIN CLOT

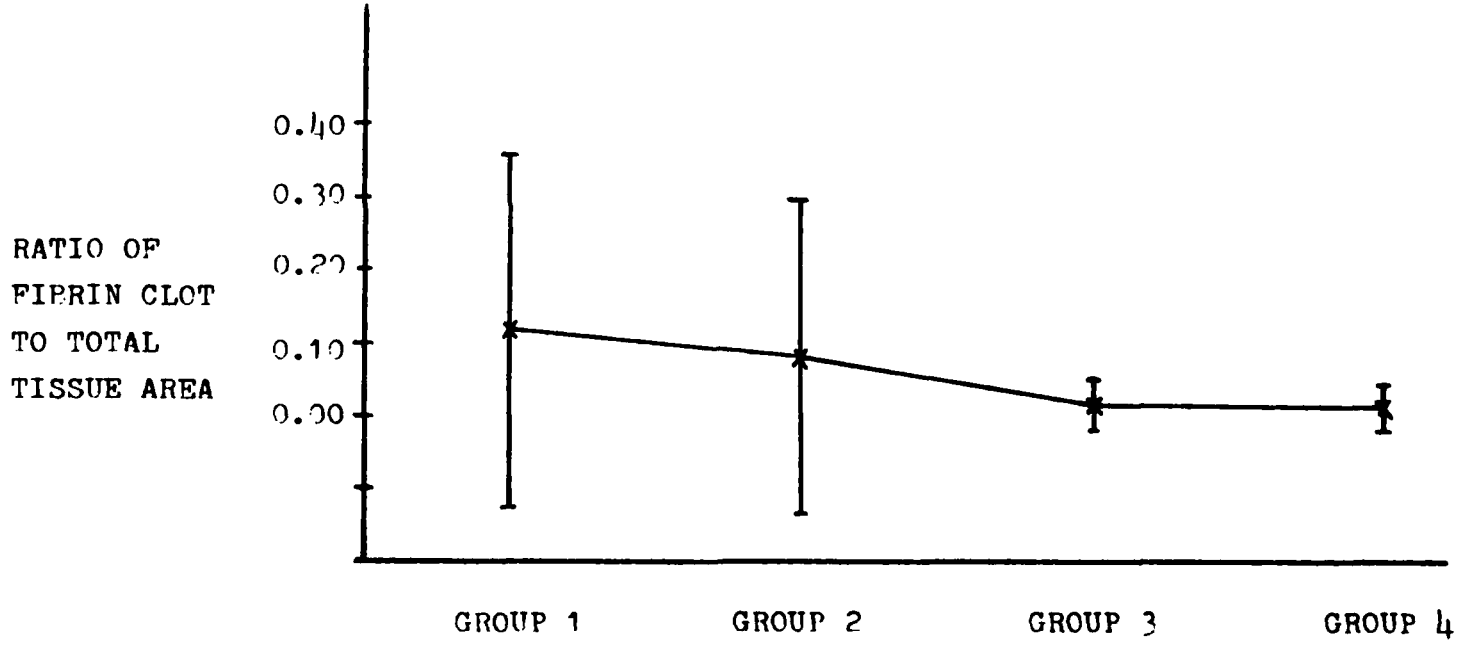


FIGURE XII

COMPARISON OF TISSUE ZONES

GRANULATION TISSUE

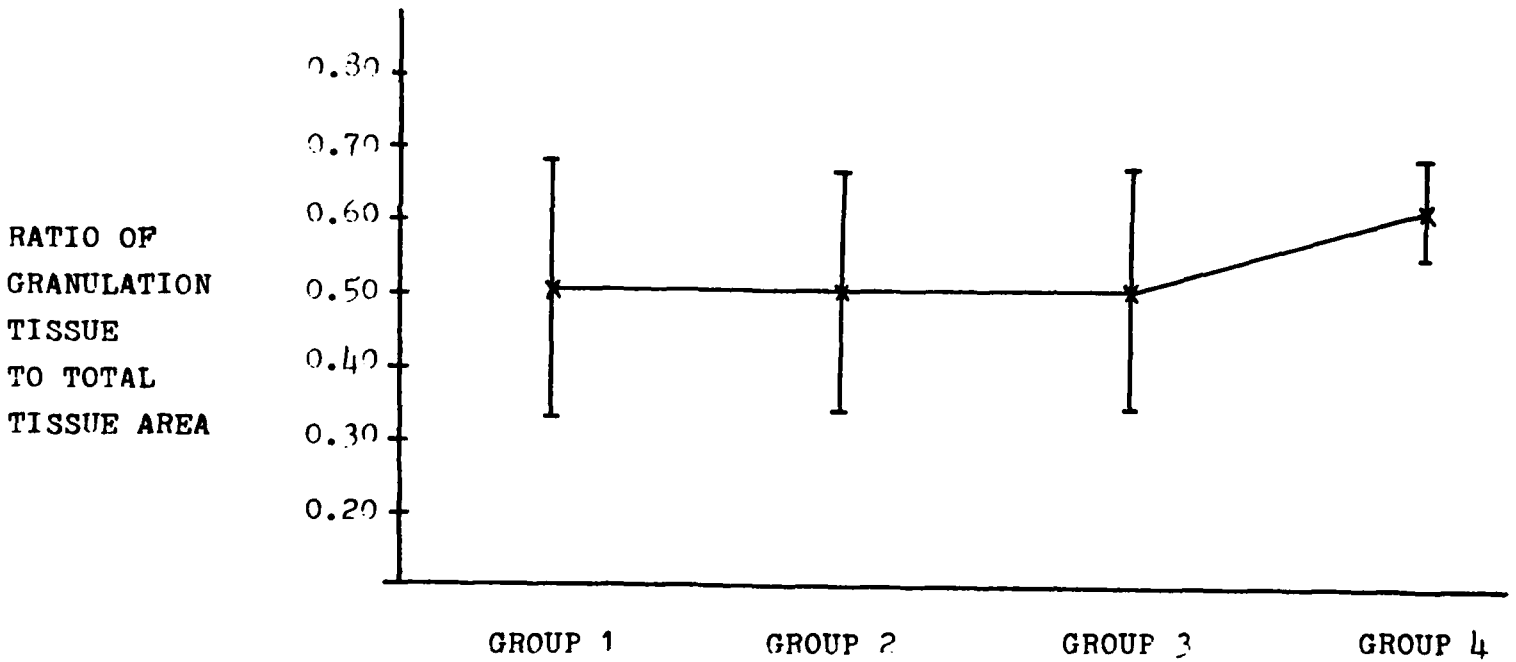
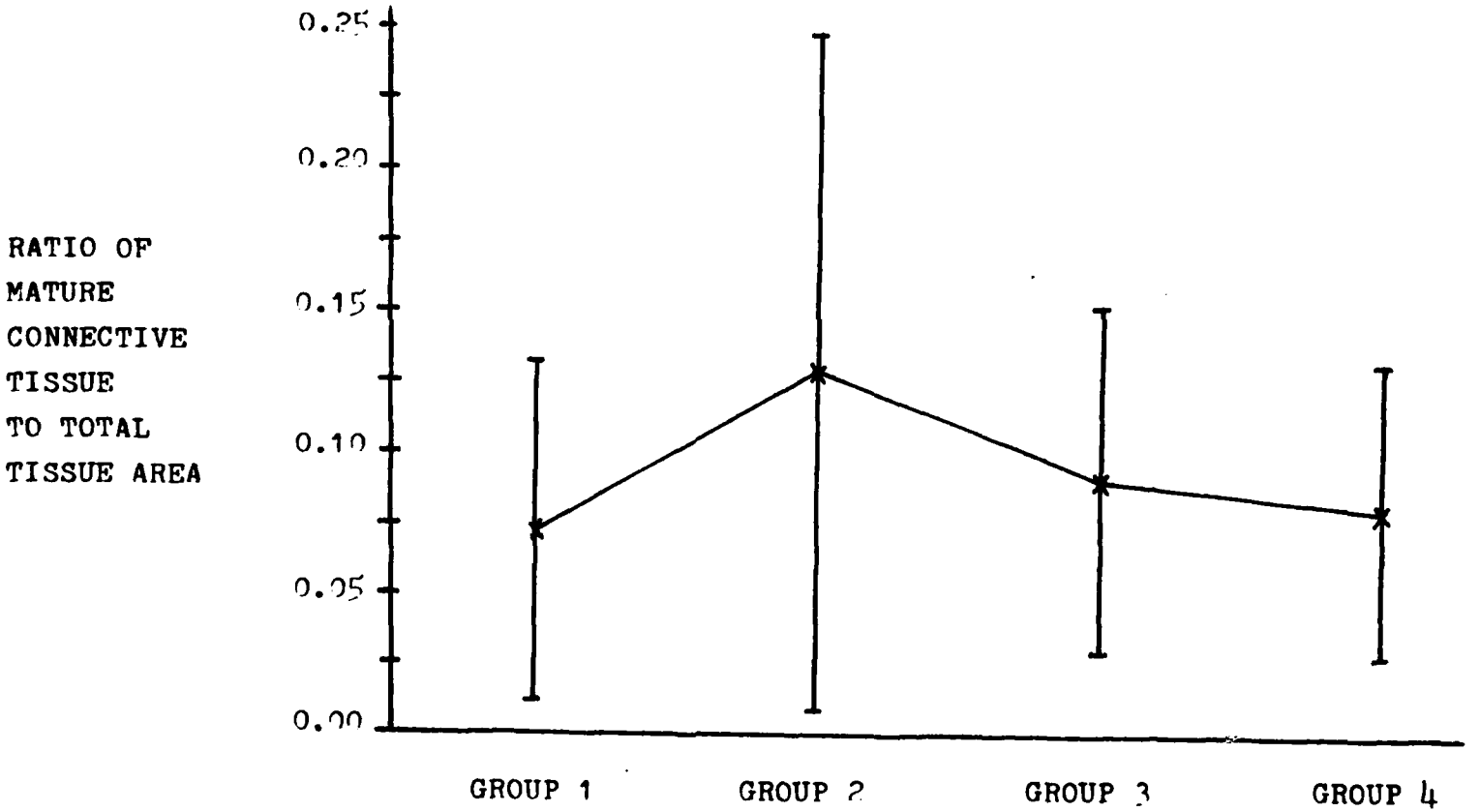


FIGURE XIII

COMPARISON OF TISSUE ZONES

RESIDUAL MATURE CONNECTIVE TISSUE



TABLES

TABLE I

MEANS AND STANDARD DEVIATIONS OF BODY WEIGHTS

DEPENDENT VARIABLE	GROUP 1 0.5 MG.(N=10)	GROUP 2 5.0 MG.(N=10)	GROUP 3 50 MG.(N=9)	GROUP 4 250 MG.(N=8)	F	df	P
BODY WEIGHTS(I)							
DAY 0 VS DAY 28	125.58 ± (II) 19.61	123.16 ± 34.16	128.78 ± 12.43	136.13 ± 24.01	0.49	3/35	.6943
DAY 28 VS DAY 35	-11.50 ± 15.93	-9.2 ± 13.34	8.89 ± 12.69	3.25 ± 9.25	5.26	3/35	.0045

(I) MEAN ± STANDARD DEVIATION
(II) WEIGHT DIFFERENCE (GM.)

TABLE II

MEANS AND STANDARD DEVIATIONS OF ORGAN TO BODY WEIGHT RATIOS

DEPENDENT VARIABLE	GROUP 1 0.5 MG.(N=12)	GROUP 2 5.0MG.(N=10)	GROUP 3 50 MG.(N=9)	GROUP 4 250 MG.(N=8)	F	df	P
ORGANS(I) KIDNEYS	.00543 ± .000343	.00544 ± .000507	.00507 ± .000200	.00492 ± .000475	2.83	3/35	.0514
SPLEEN	.00165 ± .000262	.00146 ± .000298	.00148 ± .000380	.00150 ± .000393	0.81	3/35	.5023
ADRENAL GLANDS	.000458 ± .000111	.000502 ± .000132	.000587 ± .000120	.000598 ± .000127	3.03	3/35	.0415

(I) ORGAN WEIGHT/BODY WEIGHT

TABLE III

MEANS AND STANDARD DEVIATIONS OF TISSUE ZONES
RATIO OF TISSUE TYPE TO TOTAL TISSUE AREA

DEPENDENT VARIABLE	GROUP 1 0.5 MG. (N=12)	GROUP 2 5.0 MG. (N=10)	GROUP 3 50 MG. (N=9)	GROUP 4 250 MG. (N=8)	F	df	P
EPITHELIUM	0.319 ± 0.109	0.322 ± 0.099	0.382 ± 0.145	0.285 ± 0.063	1.19	3/35	.3258
FIBRIN CLOT	0.118 ± 0.243	0.085 ± 0.216	0.017 ± 0.034	0.012 ± 0.033	0.88	3/35	.5361
GRANULATION TISSUE	0.506 ± 0.173	0.507 ± 0.164	0.511 ± 0.164	0.622 ± 0.069	1.19	3/35	.3268
RESIDUAL MATURE CONNECTIVE TISSUE	0.070 ± 0.060	0.128 ± 0.121	0.091 ± 0.062	0.081 ± 0.051	1.03	3/35	.3916

TABLE IV

SUMMARY OF SIGNIFICANT DIFFERENCES

DEPENDENT VARIABLE	INTERGROUP COMPARISONS	P
ORGAN (F=3.03, df=3/35)		
ADRENAL GLAND ORGAN TO BODY WEIGHT RATIO	1 VS 2	NS
	1 VS 3	<.05
	1 VS 4	<.05
	2 VS 3	NS
	2 VS 4	NS
	3 VS 4	NS
BODY WEIGHT (F=5.26, df=3/35)		
DAY 28 VS DAY 35	1 VS 2	NS
	1 VS 3	<.05
	1 VS 4	<.05
	2 VS 3	<.05
	2 VS 4	NS
	3 VS 4	NS

DUNCAN'S NEW MULTIPLE RANGE TEST

APPENDICES

APPENDIX A

Purina Guinea Pig Chow Pelleted
Order Number 5026
Ralston Purina Company
PO Box 548, Richmond Ind. 47374

GUARANTEED ANALYSIS

Protein, minimum	18.0%
Fat, minimum	4.0%
Fiber, maximum	16.0%
Moisture, maximum	12.0%
Ash, maximum	9.0%
Added Minerals, maximum	3.5%

CHEMICAL COMPOSITION

Nutrients:	
Protein %	18.3
Arginine %	1.08
Cystine %	.28
Glycine %	.87
Histidine %	.43
Isoleucine %	.96
Leucine %	1.46
Lysine %	.95
Methionine %	.40
Phenylalanine %	.89
Threonine %	.71
Tryptophan %	.27
Valine %	.95
Fat %	4.0
Fiber %	11.0
TDN %	67.6
NFE (by difference) %	48.2
Gross Energy, KCal/gm.	4.0

APPENDIX A CONTINUED

Ash %	8.5
Calcium %	1.1
Phosphorus %	.6
Potassium %	1.2
Magnesium %	.35
Sodium %	.32
Chlorine %	.63
Iron, ppm	298
Zinc, ppm	122
Manganese, ppm	121
Copper, ppm	21
Cobalt, ppm	2.8
Iodine, ppm	1.6
Vitamins	
Carotene, ppm	35
Thiamin, ppm	5
Riboflavin, ppm	6
Niacin, ppm	50
Pantothenic Acid, ppm	19
Choline, ppm X 100	18
Folic Acid, ppm	4
Pyridoxine, ppm	4
Biotin, ppm	0.3
B-12, mcg./lb.	6
Vitamin A, IU/gm.	30
Vitamin D, IU/gm.	3
Alpha-tocopherol, IU/lb.	20
Ascorbic Acid, mg./gm.	1

APPENDIX B

Vitamin C Deficient Diet Pelleted

Order Number 900778

ICN Nutritional Biochemicals
PO Box 28050
Cleveland, Ohio 44128

COMPOSITION:

1. SKIM MILK POWDER (BAKED) 30%
   protein 35.0%
   moisture 3.5%
   lactose 51.0%
   fat 0.8%
   ash 8.5%
   lactic acid 1.1%
   calcium 1328 mg./100 gm.
   sodium 608 mg./100 gm.
   potassium 1610 mg./100gm.
   iron 0.17%

2. GROUND ROLLED OATS 39%
   crude protein, minimum 15.0%
   crude fat, minimim 4.0%
   crude fiber, maximum 2.0%

3. GROUND WHEAT BRAN 20%
   crude protein, not less than 13.0%
   crude fat, not less than 3.0%
   crude fiber, not more than 12.0%

4. COTTON SEED OIL 8%

5. COD LIVER OIL 2%

6. SODIUM CHLORIDE 1%

APPENDIX C

VITAMIN C SUPPLEMENTS

MATERIALS

1. Double-distilled water
   Stock number 97800
   The Ohio State University
   Columbus, Ohio 43210

2. Sucrose Fame Pure Granulated Sugar
   Fame Marketing Corp.
   Dayton, Ohio 45429

3. Ascorbic Acid
   Order number 100769
   ICN Nutritional Biochemicals
   Cleveland, Ohio 44128

METHOD

Twenty percent sucrose solution was prepared by weighing 10 grams of sucrose on the Mettler H-45 analytical scale and dissolving it in 500 milliliters of double-distilled water at room temperature. The solution was stored in a labelled brown bottle. A new solution was prepared each week.

Doses of ascorbic acid supplement for each group of animals were calculated, weighed and placed in clear 20 and 30 milliliter vials. Dosage and concentration of ascorbic acid were recorded on each vial. Vial caps were marked 1 to 4 to indicate each animal group. Vials were stored at room temperature inside a box. A fresh solution was prepared each morning and supplements were given within 45 minutes of preparation.

APPENDIX C CONTINUED

GROUP I 0.5 milligram dose of ascorbic acid

Ten milligrams of vitamin C were weighed and placed into the prelabelled vials. Ten milliliters of 20% sucrose solution were added and the vial was shaken until the ascorbic acid dissolved. Each animal in Group I was given 0.5 milliliters of solution.

GROUP II 5.0 milligram dose of ascorbic acid

Seventy milligrams of vitamin C were weighed and placed into prelabelled vials. Seven milliliters of 20% sucrose solution were added and the vial was shaken until the ascorbic acid dissolved. Each animal in Group II was given 0.5 milliliters of solution.

GROUP III 50.0 milligram dose of ascorbic acid

Seven hundred milligrams of vitamin C were weighed and placed into prelabelled vials. Fourteen milliliters of 20% sucrose solution were added and the vial was shaken until the ascorbic acid dissolved. Each animal in Group III was given 1.0 milliliters of solution.

APPENDIX C CONTINUED

GROUP IV 250.0 milligram dose of ascorbic acid

Three and a half grams of vitamin C were weighed and placed into prelabelled vials. Twenty-eight milliliters of 20% sucrose solution were added and the vial was shaken until the ascorbic acid dissolved. Each animal in Group IV was given 2 supplements of 1.0 milliliter of solution, 1 half hour apart.

APPENDIX D

ANESTHESIA DOSAGE[109]

1. Preparation Formula:

ketamine hydrochloride (100 mg./cc.)	4.4 cc.
xylazine (20 mg./cc.)	2.5 cc.
sterile saline	3.1 cc.
	10.0 cc.

2. Surgical Wound Dosage

   22 mg./kg. ketamine hydrochloride

   2.5 mg./kg. xylazine

   0.5 cc./kg. dosage

3. Blood Sample Dosage

   44 mg./kg. ketamine hydrochloride

   5 mg./kg. xylazine

   1 cc./kg. dosage

APPENDIX E

BLOOD PLASMA ASCORBIC ACID TEST

Ascorbic Acid Analysis[110,111]

Reagents:

20% Metaphosphoric Acid in demineralized water

0.8% Metaphosphoric Acid in triple demineralized water (filter through 0.22 um. filter)

Normal plasma

100 mg% Ascorbic Acid in demineralized water (make fresh)

Standards:

Make standards of 2.5 ug./ml., 5.0 ug./ml., 7.5 ug./ml. using the 100 mg% stock ascorbic acid and normal plasma

Equipment:

Model 110A dual pump HPLC with a variable wavelength ultraviolet monitor set for 254 nm.

Rheodyne type injector with a 20 ul. loop

Vortex mixer

Centrifuge

C_{18} spherisorb 5 um. column

Procedure:

Pipetted 0.5 ml. sample, control or standard into a 12 X 75 test tube. Added 1.0 ml. 20% Metaphosphoric Acid, vortex and let stand for 10 minutes at room temperature.

Centrifuged for 5 minutes at 1000 X g.

APPENDIX E CONTINUED

Injected 20 ul. of supernatant onto a high performance liquid chromatographic (HPLC) column.

Plotted peak height of standards versus concentration.

Reported concentration (ug./ml.) of unknown by comparing the peak height of unknown to the standard curve.

HPLC CONDITIONS:

The mobile phase of the HPLC analysis was 0.8% Metaphosphoric Acid. The column used was a C_{18} spherisorb having an average particle size of 5 um. Twenty microliter samples were introduced into the system via syringe injection. The flow rate was 2 ml./minute. The column effluents were monitored with a 254 nm. detector.

RESULTS:

Ascorbic acid retention time was 9 minutes and recovery was approximately 100%. The coefficient variation was 5%. A linear regression curve was determined between 0.1 mg./ml. to 40 mg./ml. Sensitivity of the test was 0.1 mg./ml.

APPENDIX F

GUINEA PIG WEIGHTS (GM.)

ANIMAL	DAY 0	DAY 28	WEIGHT DIFFERENCE
	GROUP 1 (0.5 MG.)		
00	740	878	138
02	720	852	132
04	816	934	118
05	734	830	96
06	710	830	120
07	736	854	118
09	764	898	134
09	836	998	162
10	740	844	104
11	735	836	101
14	826	970	144
15	700	840	140
	GROUP 2 (5.0 MG.)		
16	742	888	146
17	724	832	108
18	812	906	94
19	686	734	48
20	694	818	124
21	886	1054	169
22	744	898	154
23	774	896	122
24	730	864	134
25	710	842	132
	GROUP 3 (50 MG.)		
27	702	836	134
28	702	822	120
29	840	944	104
30	718	844	126
31	756	884	128
32	760	902	142
33	707	830	123
34	650	790	140
35	724	872	142
	GROUP 4 (250 MG.)		
36	610	712	102
37	792	934	142
38	746	904	158
39	740	904	164
40	846	986	140
42	628	730	102
43	704	858	154
44	565	692	127

APPENDIX G

GUINEA PIG WEIGHTS (GM.)

ANIMAL	DAY 28	DAY 35	WEIGHT DIFFERENCE
	GROUP 1 (0.5 MG.)		
00	878	878	0
02	852	832	-20
04	934	932	-2
05	830	832	2
06	834	834	0
07	854	848	-6
08	898	912	14
09	998	988	-10
10	844	814	-30
11	836	794	-42
14	970	946	-24
15	840	820	-20
	GROUP 2 (5.0 MG.)		
16	858	856	-2
17	832	848	16
18	906	902	-4
19	734	700	-34
20	818	814	-4
21	1054	1042	-12
22	898	882	-16
23	896	876	-20
24	864	862	-2
25	842	828	-14
	GROUP 3 (50 MG.)		
27	836	858	22
28	822	822	16
29	944	960	16
30	844	840	-4
31	884	874	-10
32	902	928	26
33	930	950	20
34	790	798	8
35	872	874	2
	GROUP 4 (250 MG.)		
36	712	704	-8
37	934	952	18
38	904	908	4
39	904	910	6
40	988	984	-4
42	730	740	10
43	858	866	8
44	692	684	-8

APPENDIX H

ORGAN TO BODY WEIGHT RATIOS

ANIMAL	KIDNEYS	SPLEEN	ADRENAL GLANDS
	GROUP 1 (0.5 MG.)		
01	.00508	.00153	.000285
02	.00549	.00167	.000553
04	.00475	.00203	.000429
05	.00474	.00190	.000541
06	.00519	.00182	.000336
07	.00542	.00200	.000531
08	.00588	.00145	.000472
09	.00495	.00119	.000486
10	.00527	.00143	.000381
11	.00537	.00140	.000453
14	.00510	.00178	.000349
15	.00563	.00162	.000683
	GROUP 2 (5.0 MG.)		
16	.00524	.00120	.000655
17	.00513	.00197	.000507
18	.00584	.00139	.000310
19	.00607	.00159	.000729
20	.00517	.00115	.000442
21	.00485	.00151	.000336
22	.00533	.00154	.000522
23	.00473	.00155	.000525
24	.00581	.00125	.000418
25	.00613	.00111	.000580
	GROUP 3 (50 MG.)		
27	.00550	.00150	.000525
28	.00505	.00127	.000523
29	.00497	.00122	.000792
30	.00521	.00236	.000500
31	.00493	.00119	.000515
32	.00496	.00155	.000517
33	.00481	.00135	.000659
34	.00505	.00114	.000489
35	.00514	.00170	.000767
	GROUP 4 (250 MG.)		
36	.00516	.00143	.000582
37	.00490	.00169	.000588
38	.00510	.00115	.000793
39	.00527	.00170	.000615
40	.00454	.00149	.000539
42	.00553	.00228	.000757
43	.00400	.00113	.000497
44	.00487	.00116	.000409

APPENDIX I

ASCORBIC ACID PLASMA ANALYSIS

ANIMAL	ASCORBIC ACID PLASMA LEVEL (UG/ML)
GROUP 1 (0.5 MG.)	
00	0.0
02	0.0
04	0.0
05	0.0
06	0.0
07	0.0
08	0.0
09	0.0
10	0.0
11	0.0
14	0.0
15	0.0
GROUP 2 (5.0 MG.)	
16	0.0
17	0.0
18	0.0
19	0.0
20	0.0
21	0.2
22	0.4
23	0.0
24	0.3
25	0.8
GROUP 3 (50 MG.)	
27	0.0
29	0.7
29	0.0
30	0.0
31	0.0
32	0.2
33	0.0
34	0.0
35	0.4
GROUP 4 (250 MG.)	
36	0.0
37	0.0
38	0.0
39	0.0
40	0.0
42	0.0
43	0.0
44	0.0

APPENDIX J

GUINEA PIG WOUND MEASUREMENTS

RATIO OF TISSUE TYPE TO TOTAL TISSUE AREA

GROUP 1 (0.5 MG.)

ANIMAL	EPITHELIUM	FIBRIN	GRANULATION	RESIDUAL MATURE CONNECTIVE TISSUE
00	.254	0	.718	.028
02	.174	.686	.140	0
04	.123	.568	.309	0
05	.375	0	.484	.141
06	.521	0	.324	.155
07	.438	.013	.436	.113
08	.301	0	.644	.055
09	.267	.150	.567	.167
10	.319	0	.639	.042
11	.314	0	.658	.028
14	.344	0	.581	.075
15	.397	0	.569	.034

APPENDIX J

GUINEA PIG WOUND MEASUREMENTS

RATIO OF TISSUE TYPE TO TOTAL TISSUE AREA

GROUP 2 (5.0 MG.)

ANIMAL	EPITHELIUM	FIBRIN	GRANULATION	RESIDUAL MATURE CONNECTIVE TISSUE
16	.167	.691	.143	0
17	.303	.012	.636	.455
18	.360	.116	.395	.128
19	.190	0	.667	.143
20	.368	0	.539	.092
21	.440	.027	.413	.120
22	.479	0	.425	.096
23	.268	0	.659	.073
24	.304	0	.609	.087
25	.337	0	.581	.081

APPENDIX J

GUINEA PIG WOUND MEASUREMENTS

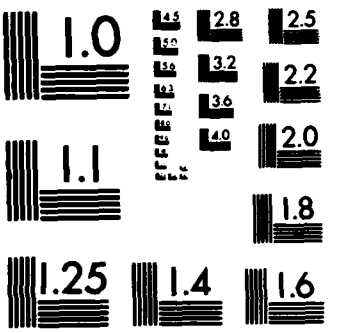
RATIO OF TISSUE TYPE TO TOTAL TISSUE AREA

GROUP 3 (50 MG.)

ANIMAL	EPITHELIUM	FIBRIN	GRANULATION	RESIDUAL MATURE CONNECTIVE TISSUE
27	.255	.091	.636	.018
28	.302	0	.619	.079
29	.423	0	.519	.058
30	.449	.058	.464	.029
31	.716	0	.119	.135
32	.315	0	.539	.146
33	.229	0	.711	.060
34	.365	0	.554	.081
35	.385	0	.407	.209

AD-A132 481 THE EFFECTS OF VARIOUS LEVELS OF ASCORBIC ACID INTAKE UPON ORAL WOUND HEALING IN GUINEA PIGS(U) AIR FORCE INST OF TECH WRIGHT-PATTERSON AFB OH A E STANYA 1983 2/2

UNCLASSIFIED AFIT/CI/NR-83-29T F/G 6/5 NL



APPENDIX J

GUINEA PIG WOUND MEASUREMENTS

RATIO OF TISSUE TYPE TO TOTAL TISSUE AREA

GROUP 4 (250 MG.)

ANIMAL	EPITHELIUM	FIBRIN	GRANULATION	RESIDUAL MATURE CONNECTIVE TISSUE
36	.222	0	.667	.111
37	.275	0	.652	.072
38	.160	.093	.733	.013
39	.329	0	.575	.096
40	.333	0	.625	.042
42	.315	0	.528	.157
43	.319	0	.653	.023
44	.327	0	.545	.127

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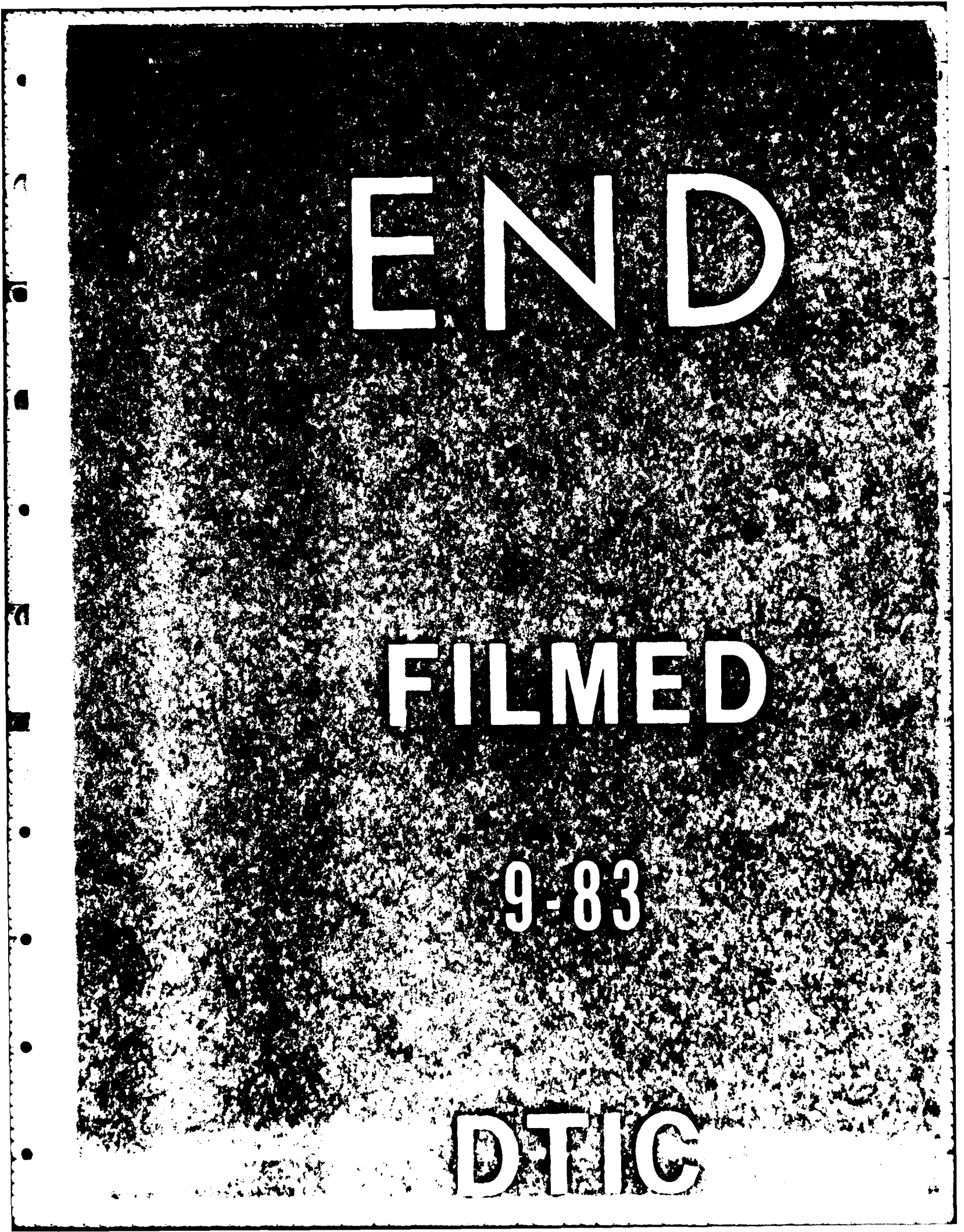
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