THE EFFECT OF NUTRITIONAL ANEMIA ON THE TENSILE STRENGTH OF HEALING SOFT TISSUE AND BONE WOUNDS IN THE RAT

By

Robert Leon Ewbank

Submitted to the Faculty of the Graduate School in partial fulfillment of the requirements for the degree, Master of Science in Dentistry, in the Department of Oral Pathology, Indiana University, 1961.

ACKNOWLEDGEMENTS

ACKNOWLEDGEMENTS

I would like to thank all the people who were so helpful while this project was being completed.

Special acknowledgement is given to Doctor William G. Shafer who was so generous with his time and instruction. It was an honor to have had Doctor Shafer as my counselor and as an instructor during my undergraduate and postgraduate training. To Doctor Shafer I am greatly indebted.

I would like to thank Doctor S. Miles Standish for his advice and encouragement during the many hours of preparation.

Miss Patricia G. Clark, H. T. (A.S.C.P.) is to be richly thanked for her advice and help in the biological laboratory procedures. Her valuable assistance was greatly appreciated.

I would like to thank Mrs. Naomi Ress and Kathy Kuntz for their untiring efforts in preparing the histological material.

Mrs. Gloria Spray and Mr. Richard Scott did the excellent photographic preparations and are to be highly praised for their work.

The assistance given by the staffs of the Dental and Medical libraries was greatly appreciated.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (Continued)

INTRODUCTION

INTRODUCTION

Many aspects of wound healing have been studied experimentally in both animals and humans in considerable detail, but the influence of nutritional anemia on wound healing has had little mention in the medical or dental literature.

It has been the clinical impression of some surgeons[1, 2] that wounds in anemic patients heal poorly. The few controlled studies which are found in the literature, however, appear to be inconclusive as far as determining whether a nutritional anemia does have an adverse effect on wound healing in experimental animals. It was felt, therefore, that the problem of nutritional anemia and its relationship to experimental wound healing should be further investigated.

REVIEW OF THE LITERATURE

REVIEW OF THE LITERATURE

Anemia is recognized as a reduction in the number of erythrocytes or a reduction in the amount of hemoglobin per unit volume of circulating blood. It may result from a great many causes and is often accompanied by various changes in the appearance of erthrocytes. The common classifications of anemia are based either on the structural changes in the erythrocytes or on the etiology and pathogenesis of the disease.

If an adequate amount of iron is not available for hemoglobin formation, a microcytic hypochromic type of anemia will develop. Beutler[3] presumed that the primary defect is in the synthesis of the prosthetic group of hemoglobin, heme. Therefore, he carried out a study to determine the iron content of hemoglobin in iron deficiency. He concluded from his findings that in iron deficiency a defect in the heme synthesis appears to limit in some manner the synthesis of globin.

In iron deficiency anemia, there is a reduction in the blood hemoglobin level which is proportionately greater than the reduction in the number of erythrocytes. The mean corpuscular hemoglobin concentration is found to be below normal. The hematocrit reading is low and this reduction in the volume of the packed erythrocytes is also proportionately greater than the reduction in the number of erythrocytes. A low volume index results and the mean corpuscular volume is found to be decreased, an indication that the average size of the erythrocytes

is less than normal[4].

Schwartz and Flowers[5] found that the development of iron deficiency anemia is characterized by (1) a linear fall in the hemoglobin and (2) a fall in red blood cell count which lags behind the hemoglobin fall and results in a progressive diminution in color index. At a hemoglobin level of about 25 per cent (3.9 grams), the color index reaches its minimum (about 0.5) and, from this point on, the fall in hemoglobin value and the fall in red blood cell count parallel each other. Characteristic morphologic changes occur in the red blood cells with the fall in color index in the following order: microcytosis, hypochromia, anisocytosis, and poikilocytosis.

Experimental Production of Anemia.

Mitchell and Schmidt[6] have shown that a true nutritional anemia can be produced in rats born to parents fed diets low in iron. The red blood cell counts of the anemic rats ranged from 1.3 to 4.9 million per cubic millimeter.

Waddell, Steenbock, Elvehjem, and Hart[7] reported in 1928 that young rats produced in their stock colony, when weaned at the age of 3 to 4 weeks and placed on a whole (cow) milk diet, developed a profound anemia in 6 to 8 weeks. They found that the hemoglobin levels were reduced from a level of 10.8 grams per 100 cubic centimeters of whole blood to 4 grams or less. They noticed that the rats grew well for a period of 4 to 6

weeks on the milk diet, but after this time the weight generally became stationary and finally a rapid decline occurred. After eight weeks, death rapidly ensued.

Elvehjem and Kemmerer[8] reported an improved technique for the production of nutritional anemia in rats by allowing a pregnant rat no dry food in the cage, but feeding whole (cow) milk ad libitum. The mother was removed to a separate cage every day for the feeding of the dry ration and returned to the young after being thoroughly brushed to remove any adhering food. By this procedure, the young obtained only the mother's milk until they were 12 days old. After their eyes were opened, they also consumed some of the milk, but they came into contact with no other food. The mother was removed when the young were 21 days of age. A severe anemia developed within 2 weeks after weaning. The weight of the animals decreased during the second and third weeks, and very few rats lived past four weeks. They found that the hemoglobin content of the blood during the second week was generally between 2.0 and 3.5 grams. The rats seldom survived more than a few days when the hemoglobin reached a level of 2.0 grams.

Robscheit-Robbins and Whipple[9] found that in dogs the hemoglobin production was increased as the anemic condition became more severe.

Smith and Medlicott[10] have stated that a deficiency of iron or copper or both produces an anemia in the rat that morpholog-

ically may be classified as microcytic and hypochromic.

Cunningham[11] suggested that the anemia that develops in rats fed a diet of cow's milk possibly may be due, in part, to Bartonella infection. Newly weaned rats (3 to 4 weeks old) restricted to such a diet consume only about 30 milliliters of milk daily which is approximately 4 grams of solid material per day compared to 10 to 15 grams per day consumed by rats on a solid diet. This inadequate caloric value of the food along with the susceptible age of the rats produce a set of conditions favourable to the development of Bartonella muris infection.

Gorter[12] in 1933 attempted to establish the importance of copper deficiency by determining the copper content of the blood in patients with nutritional and other forms of anemia. He felt that if copper were one of the causative factors in nutritional anemia, then the copper content of the blood should be lower. However, just the reverse was found. He reported that human patients with nutritional anemia have a higher level of copper in the blood than did individuals without nutritional anemia of the same age. He stated that "the production of hemoglobin requires a certain amount of copper and its increased production in anemia requires more copper. If it is not present, then the hemoglobin production can not take place".

In 1935 Elvehjem[13] carried out an excellent review of the literature on copper and its relation to iron metabolism. He stated that copper is necessary as a supplement to iron for

hemoglobin formation in red blooded animals. Copper is not concerned with the assimilation of iron, but rather with the transformation of the ingested iron into hemoglobin. He mentioned that milk has a low concentration of copper and iron.

Schultze, Elvehjem, and Hart[14] reported studies on the copper content of the blood in nutritional anemia in pigs. They found that hemoglobin and erythrocyte formation does not take place in spite of the high copper content of the blood if iron is lacking, and the anemia will persist. They also noted that pigs suffering from a nutritional anemia due to iron and copper deficiency, exhibited a decrease in the copper content of the blood which falls to an extremely low level, but that the feeding of 2 to 4 milligrams of copper per day with iron results in a very rapid and significant increase of the copper content of the blood. They felt that rapid continuous hematopoiesis can not take place unless the copper content of the blood is maintained above a minimum level.

Cunningham[15] has shown that copper deficient heifers have a high incidence of bone fractures, usually the humerus. The fractured bones showed a very mild degree of osteoporosis, but were virtually normal in appearance and showed no macroscopic defect that would account for their apparent brittleness. He also observed a definite osteoporosis in lambs that had been fed a copper deficient diet. The mechanism of this bone change is unknown.

Teague and Carpenter[16] produced copper deficiency in growing pigs and noted, in addition to a dietary anemia, that a deformity of the leg occurred. There was a lack of rigidity in the leg joints and the hocks became excessively flexed, forcing the animal to assume a sitting position. The forelegs showed various degrees and types of crookedness. Therapeutic cases of copper were shown to be effective, and in some cases brought about a complete reversal of symptoms.

Lahey, Gubler, Chase, Cartwright, and Wintrobe[17] noted that the anemias which developed in copper deficient and iron deficient swine were very similar morphologically, as well as biochemically. However, they mentioned finding skeletal abnormalities in the animals which were copper deficient, but made no mention of this fact in iron deficient animals.

In a microscopic study of copper deficient dogs by Baxter, Van Wyk, and Follis[18] there was found an excessive resorption of bone, particularly around the inner portions of the cortex and also in the shaftward portions of the metaphysis. There was also decreased deposition of bone matrix. These changes did not appear to be related to the bone marrow hyperplasia. They concluded that the bone disorder consisted of a diffuse osteoporosis with no primary disturbance in the calcification mechanism.

Baxter, and Van Wyk[19] found that young dogs made severely copper deficient developed a bone disorder characterized by

abnormally thin cortices, deficient trabeculae and wide epiphyses. Anemia and graying of the hair also occurred. Deformities and fractures of the bones occurred in many of the copper deficient animals. The disorder was relieved by the administration of copper and did not occur in the control animals. Iron deficiency with severe anemia did not produce similar bone changes, apparently indicating that the bone disorder is a specific effect of a copper deficiency.

Van Wyk, Baxter, Akeroyd, and Motulsky[20] noted that dogs anemic, due to an iron deficiency, had the classical changes in peripheral blood consisting of relatively normal numbers of red blood cells, but a marked decrease in red cell size, hemoglobin content and concentration. In the bone marrow, iron deficiency resulted in hyperplasia of hemoglobin deficient normoblasts with no appreciable shift to the left or evidence of a disorder of maturation. In contrast to the anemia of iron deficiency, the anemia which occurred as a result of copper deficiency was characterized by a reduction in the number of erythrocytes, with the maintenance of relatively normal red cell indices. In the marrow there was no evidence of a deficiency in hemoglobin content, but evidence rather of a defective development of cells of the erythrocytic series. They emphasized that copper is essential for normal maturation of the erythrocytic elements of the marrow and production of normal numbers of red blood cells.

Follis, Bush, Cartwright, and Wintrobe[21] carried out microscopic examination of bones of swine which were copper deficient and found that there was a marked reduction in osteoblastic activity as evidenced by a failure of bone to be deposited on the calcified cartilage matrix; furthermore, the latter is not resorbed in the normal fashion. In contrast, growth of cartilage is not affected. It would appear that thinning of the cortex is due to decreased osteoblastic activity as well.

The Effect Of Anemia On Wound Healing.

In 1943 Besser and Ehrenhaft[22] studied the tensile strength of stomach wounds in dogs. They used 26 control and 26 experimental animals which were made acutely anemic pre-operatively by bleeding. Intravenous pooled plasma was given at the time of bleeding to replace the lost fluid. Daily determinations of tensile strength were carried out and it was found that the hematocrit had returned to the normal level by the eleventh day. They concluded that acute anemia in dogs does not retard wound healing as judged by tensile strength and microscopic study.

Sandblom[23] used rabbits in another study of the effect of anemia on wound healing. Skin wounds were produced before the animals were bled, and the tensile strength tested from 5 to 7 days after the wounding. New wounds were then placed in the same animals after they had been bled. It was found that with acute anemia there was a decrease of 39.2 per cent in tensile

strength of the wounds. In the animals with control wounds the hemoglobin value was 78 per cent, and in the animals with experimental wounds the hemoglobin was 35 per cent.

Waterman, Birkhill, and Levenson[24] produced nutritional anemia in albino rats by maintaining them on an unsupplemented diet of ordinary pasteurized cow's milk. One group of control animals was fed milk plus iron diet and another group stock laboratory diet. Hemoglobin and serum protein studies were performed on all animals. The anemic animals had a hemoglobin level of 5 to 7 grams. Three centimeter midline laparotomy incisions were made on 130 animals, and the tensile strength tested at 3, 5, 7, 9, and 11 days post operatively. They tested the tensile strength of the wounds by inflating, at a fairly constant rate, an intra-abdominally placed condom until the wound disrupted. They concluded that there was no significant difference between the means of the tensile strength of the wounds of the anemic, non-anemic milk plus iron, and the control animals of the same weight as the milk-fed rats.

Sako, Kremen, and Varce[25] measured the healing strength of standard wounds in anemic and starved animals and found that, after treatment with blood transfusions or refeeding, the tensile strength of the wounds increased virtually to the control normal values. This study demonstrated that an impairment of wound healing, secondary to either acute or chronic anemia, is correctable by transfusion of either fresh or banked blood at,

or immediately after, the standard operation.

General Healing Of Incised Soft Tissue Wounds.

Harvey[26] studied the velocity of growth of fibroblasts in healing wounds of rat's stomachs and found that the tensile strength of a healing wound was a function of the multiplication and maturation of the fibroblasts. He also noted that there is a latent period of about 4 days after infliction of the wound before fibroplasia becomes appreciable. In 1930 Harvey and Howes[27] tested the tensile strength of healing wounds of rat's stomachs to determine the effect of a high protein diet on the velocity of the growth of fibroblasts in these healing wounds. From their study they felt that, once growth has started, the velocity of the fibroblasts distinctly increase by a high protein diet. They felt that the maximum strength of a wound may be reached some 2 days earlier than in animals fed a standard diet.

In 1932 Howes and Harvey[28] studied the age factor in relation to the velocity of the growth of fibroblasts in the healing wound and found that healing in the young is more rapid than in adults, because the rate of fibroplasia is greater. They presented curves to substantiate their findings that there is a shorter lag period in developing tensile strength with young rats and the tensile strength is greater in the young animals.

A year later Howes, Briggs, Shea, and Harvey[29] studied the

effect of complete and partial starvation on the rate of fibroplasia in the healing wounds of rat stomachs. They carried out daily tensile strength tests and found that the healing strength of wounds produced in the stomachs of adult rats was not appreciably affected by complete starvation. The wound tensile strength in the experimental adult rats was not affected by feeding them one-half the amount of diet given the control animals. On the other hand, the healing of wounds in the stomachs of young rats was decidedly retarded by restricting their diet to only one-half that amount given the control animals. The investigators felt that the retardation of the healing wound in the young rat could possibly be due to the fact that there was not enough food element or elements capable of stimulating and maintaining cellular proliferation during this early age. The adult rat stomachs healed in spite of great variations in the amount of food consumed.

In 1935 Howes and Harvey[30] pointed out that, so far as the function of tensile strength is concerned, we are dealing primarily and essentially with the growth and maturation of the fibroblast. They stated that fibroplasia starts abruptly at the fourth day and proceeds with decreasing velocity until a maximum strength for this phase of healing is reached at the 12th to the 14th day. By the 6th day approximately one-third, and by the 8th day approximately two-thirds of this strength is obtained. They felt that wound healing is only disturbed by a profound systemic change. No attempt was made to study the effects of anemia on wound healing.

Smelo[31] approached the problem of wound healing by attempting to determine the effects of local agents. He used a variety of materials, such as antiseptics, stimulants, and inert materials, and found through a method of quantitative evaluation that there was no effect of local agents on the velocity of wound repair. Factors other than the local dressing appeared to play the dominant role in determining the rate of wound healing.

Lanman and Ingalls[32] studied the effects of vitamin C deficiency on the tensile strength of wounds and found that, in the scorbutic group of animals, tensile strength averaged only one-third that of the normal group.

Taffel and Harvey[33] have suggested that the lack of vitamin C may be the causative factor in those cases of wound disruption in humans where there is no evidence of infection, and where the patients have been on an inadequate dietary regime. Partial starvation, as manifested by a loss of body weight, seemed to have no effect on the progress of healing in the control.

Burr, Harvey, and Taffel[34] felt that the determination of tensile strength must be concerned with at least 2 processes, one of cell proliferation, and one of cell differentiation. In normal development, the two events do not occur in the same cell simultaneously. Rather, each cell takes part in the mitotic activity of a group of cells for a period of time after which it undergoes, with other cells, a period of differentiation. As growth proceeds, new cells enter mitosis and then differentiate adding thereby to the new structure.

In 1938 Thompson, Ravdin, Rhoads, and Frank[35] studied the effect of hypoproteinemia on wound disruption in the abdominal wall of dogs. They controlled all possible variable factors except hypoproteinemia, but disruption of the wounds still occurred in a large number of the wounds. The failure of the wounds to heal was believed to be associated with the hypoproteinemic state.

The relation of protein deficiency to experimental wound healing in the rat also has been studied by Kobak, Benditt, Wissler, and Steffee[36] in 1947. Their results led them to believe that protein deficiency hampers wound healing from the third through the fifth post operative days. It is apparently due to a diminution in the number of fibroblasts, a decrease in their rate of maturation, and a general failure to organize with adequate density along the lines of stress. This leads to a delay in maturation and transformation of the reticulum into mature collagen. The wounds also tend to heal with more infection.

Charney, Williamson, and Bernhart[37] carried out determinations of tensile strength of healing wounds in rats and found that their data confirmed the report of Harvey and Howes[27] on the beneficial effect of high protein diets on the healing of experimental wounds.

Fendlay and Howes[38] suggested from their experiments that edema per se does not interfere with fibroplasia and wound healing, if the protein needs of the cells are adequately supplied.

Williams, Mason, and Bradshaw[39] have shown that secondary wounds in rats with normal plasma proteins gain in tensile strength more rapidly than primary wounds in the same animal. The over-all strength of wounds in hypoproteinemic rats is less than of those in animals with normal plasma proteins. However, the secondary wound in hypoproteinemic animals is stronger during the first six days of healing than is the primary wound in animals with a normal plasma protein during the same time.

In 1939 Lawrence, Pearse, and Mider[40] studied the effect of injecting antileukocytic serum into guinea pigs and the relationship to wound healing. They felt that the essential feature of repair is fibroplasia, and the polymorphonuclear neutrophil plays a part in the repair of wounds only so far as it helps to combat infection.

Burr, Harvey, and Taffel[41] carried out an electrometric study of the healing wound in man and found that the immediate reaction to injury and the subsequent reparative process induced by an incised wound as measured by its tensile strength always occurred in a consistent manner. The phase in which the exudative reaction predominates was represented in their curve as a "lag period" of about four days, during which there was no material increase in the tensile strength. This was followed by the proliferative phase, histologically marked by a rapid increase of fibroblasts with an increase in tensile strength which near the tenth day causes the curve to become asymptotic.

In 1940 Preston[42] reported the effects of sutures on the strength of healing wounds in rats, noting that wounds closed with annealed stainless steel wire (35 Band S gauge) possessed the greatest average strength and showed the least local reaction to the suture material. Wounds that were closed with number 0 plain catgut were weakest and showed the greatest local reaction to sutures. Gross infection was not present in any of the wounds closed with wire, but was present in some of the wounds closed with silk, chromic catgut, and plain catgut. He also noted that interrupted loose stitches placed near the wound resulted in the strongest skin wounds. Continuous tight stitches were found to produce the weakest skin wounds.

In 1943 Localio, Casale, and Hinton[43] studied the effect of infection on wound healing in rats. They operated several hundred rats with a sterile technique, and performed cultures for aerobic organisms. They found that 20 per cent of the catgut-sutured wounds were positive for organisms, but only 7.1 per cent for silk, 7.8 per cent for wire, 4.7 per cent for cotton, and 7.3 per cent for nylon. They also found that microscopically there were marked differences between the catgut and non-absorbable suture materials. In catgut there was seen an acute intense prolonged inflammatory reaction associated with widespread death of tissue and abscess formation. The delay in final healing of catgut-sutured wounds, as noted in studies of tensile strength and also microscopically, must be attributed to the

more widespread destruction and acute inflammatory reaction of the tissues in these wounds. Their conclusions were that non-absorbable sutures are superior to absorbable sutures with respect to tensile strength and that in this same respect, one non-absorbable suture is not to be preferred over another. They felt that an increase in tensile strength associated with a particular type of suture material should be taken into consideration for clinical application. They found no real basis for preferring one non-absorbable suture over another as far as tensile strength of wounds during the first 5 post-operative days was concerned. Both non-absorbable sutures resulted in stronger wounds than did catgut sutures.

Chassin, McDougall, MacKay and Localio[44] studied the effect of stress upon the healing of wounds in rats and found that there was no significant difference between bursting pressure of control and experimental animals.

Savlo, and Dunphy[45] showed that disrupted and resutured wounds acquired more strength by the third day than primary three day wounds. They found that cortisone in large doses started before wounding retarded healing, and abolished the accelerated rate of healing in three day resutured wounds. However, if cortisone were started at the time of disruption of the primary wound, it did not inhibit the accelerated healing of the resutured wound. They stated that local factors are responsible for faster healing in resutured wounds, although the nature of these remains to be determined.

Borgstrom and Sandblom[46] studied suture technique and wound healing in rabbits and found that, with the same degree of tissue tension, there was no difference in wound healing whether interrupted sutures or continuous sutures were used. There was not a statistically significant difference in wound healing except when wounds with and without tissue tension were compared. This comparison showed that wounds closed with tension have a lower tensile strength.

Shafer, Beatty, and Davis[47] studied the effect of dilantin sodium on healing wounds in 1958, and found that systemic administration of the drug produced a dramatic increase in tensile strength of such wounds in rats. They felt that this probably was a function of increased collagenization, the same basic reaction which accounts for the undesirable side reaction of gingival hyperplasia in epileptics receiving the drug. The mechanism of action of the dilantin sodium on wound healing is unknown.

Nishihara and Prudden[48] found from a study on rats that transverse incisions are considerably stronger than midline incisions. They found that there is no appreciable decrease in wound tensile strength when the abdominal incisional length was increased from 50 to 100 per cent of the maximal possible length. They, therefore, suggested that incisions for major abdominal operations should be made as long as is necessary to eliminate completely any interference with exposure referable

to incisional length since such a practice will result in no significant decrease in wound strength. They also noted that post-operative external abdominal support decreased the inherent tensile strength of wounds.

General Healing Of Bone Wounds.

Lindsay and Howes[49] studied the breaking strength of healing fractures in rats and concluded that the strength of the bone first manifests itself by the sixth day and increases rapidly to the twenty-first day. A period of decline in strength occurs after the twenty-first day, during and following which time the callus decreases. There is a secondary rise in strength from the thirty-third to forty-fifth day.

McKeown, Lindsay, Harvey, and Howes[50] found that the breaking strength of healing fractures in rat fibulae was positively correlated with fibular length and animal weight.

The effect of insulin on the healing of experimental fractures in the rabbit was studied by Stuck[51] in 1932. He felt that healing of fractures was an essentially local process, involving local vascular changes and redistribution of calcium. He also felt that insulin had no clinical application in bone healing, even though he found a slight increase in the rate of calcification of the callus in the rabbit.

Gregory[52] studied the effect of systemically administered protamine-zinc insulin on the healing of both bone and soft tissue wounds and found that there were no differences in the

degree of healing between the control and experimental groups which could be detected by histologic study, roentgenographic study, or by breaking strength tests.

Bell, Cuthburtson, and Orr[53] studied the strength and size of bone in relation to calcium intake, and found no harmful effects occurred in rats maintained on a diet containing more than 0.36 per cent calcium, but there was apparently no advantage of such a high intake.

In 1947 Bell, Chambers, and Dawson[54] studied the mechanical and structural properties of bone in rats on a rachitogenic diet. They found that there is no disturbance of the fundamental plan of ossification in rats suffering from rickets. "The weakness of the rachitic bone is sufficiently explained by the reduction in inorganic material relative to the organic material."

Bourne[55], in 1942, showed that the deposition of bone salt in normal and regenerating bone is retarded in scurvy, but pure synthetic vitamin C permits this process to take place. "Vitamin C may play some part in the formation or stabilization of alkaline phosphatase."

Bell and Cuthbertson[56] studied the effect of various hormones on the chemical and physical properties of bone in 1943. They found that the bones from rats treated with extracts of the anterior pituitary, parathyroid and oestradiol dipropionate were proportionately larger and stronger than those from the controls; the reverse was true of the femurs in animals administered thyroid.

However, the difference in the quality of the bone as indicated by the breaking strength of the experimental and control animals was slight. They concluded that the differences in the breaking strength were the result of changes in the dimensions of the bones rather than in the quality of the bones. The latter characteristic, as indicated by the slight differences in the breaking strength of the bones, was not changed by the hormones administered to the animals during the experiments.

Gardner[57] found that estrogens were associated with the breaking strength of mice femurs, producing stronger bones in treated animals. He also noted that testosterone decreased the breaking strength.

Deaton, Forsyth, Coppedge, and Bradshaw[58] studied the effects of sex hormones in the healing fractures of dogs. They found that sex hormones have no demonstrable effects on experimental fracture healing in healthy adult dogs as determined by evaluation of the roentgenographic changes, pertinent blood chemistry findings, weight fluctuations and breaking strengths.

Eskelund and Plum[59] studied the effect of extracts of thymus, lymphatic, splenic, and adrenal tissue and found that the thymus extracts contain a factor which when injected intramuscularly in adequate doses distinctly accelerated healing fractures. Their results with lymphatic and adrenal tissue were not comprehensive enough to permit a definite conclusion. Splenic tissue was not effective in accelerating healing fractures.

Gillespie[60] investigated the nature of bone changes associated with nerve injuries and disuse. He tested the ultimate bending stress of bones from kittens in the same fashion as described by Bell, Cuthbertson and Orr[53]. He noted that the average ultimate bending stress of the bones was greater in the denervated limb in which the anterior and posterior spinal roots had been sectioned. He concluded that the altered changes in the physical properties, including ultimate stress, of bones from the paralyzed limbs was almost entirely due to a decrease in the quantity of bone resulting from secondary loss of muscle activity. He found no evidence that vascular changes were involved or that nerves exert any specific trophic influence on bone.

Savchuck[61] studied the effects of strontium and fluorine on the repair of unreduced humeral fractures in the adult rat and found that there was no significant effect upon the breaking strength of the fractured humerus.

Fahim[62] carried out a histologic study of bone repair in lathyritic rats and found that if aminoacetonitrile were administered to rats with experimental bone wounds, there was an inhibition of bony union.

Methods of Testing Tensile Strength of Soft Tissue Wounds.

In 1929 Howes, Sooy, and Harvey[63] produced incisions in the skin, muscle, fascia, stomach, and intestines of dogs, sutured

the wounds and tested their tensile strength at various time intervals. At the time of testing, the tissue containing the wound was removed from the animal, the length of the wound determined, and its tensile strength determined on a Scott thread-testing machine.

Harvey[26] approached the problem of testing tensile strength in another manner during the same year. He made incisions in the stomachs of rats, sutured the wounds, and also tested their strength at various periods. At the time of testing, the esophagus was ligated and a cannula inserted into the pyloric area. The cannula was connected to a source of air pressure. Air was admitted at a uniform rate until the stomach wound ruptured. The pressure of air was measured by a mercury manometer recording on a revolving drum.

Harvey and Howes[27] used the same procedure in 1930 and concluded that this technique does not give an absolute value of the strength of the stomach or wound but inasmuch as the data desired are that of the relative change in strength, the method seemed adequate.

Ellis[64] devised an apparatus in 1931 to test the tensile strength of wounds made with a steel knife blade versus wounds made electrically. The apparatus consisted of a tensiometer with an arm balance devised to pull the wound apart. The tissue was removed from the body and one end of the tissue was clamped in a stationary position while the other end of the tissue was clamped to a wire cable which ran over a wheel to the arm of

the balance. Weight was then added to the balance until the wound disrupted.

In 1937 Burr and Hovland[65] used the Burr-Lane microvoltmeter to determine with reasonable certainty bio-electric potential differences in the living organism. They showed that it was possible to study changes in the gradient with time and, thereby, use it as a measuring scale for growth in the living organism. In 1938 Burr, Harvey and Taffel[66] used the Burr-Lane microvoltmeter to record at frequent intervals the growth processes of a wound without interrupting its healing. They marked points on guinea pig flanks and measured e. m. f. between two points. This was performed on incised areas as well as on non-incised areas. The data showed a marked change in the potential gradients between the normal skin and the area of injury and by these means were able to plot the rate of healing of a wound.

Lanman and Ingalls[32] devised an apparatus with a mercury sphygmomanometer, the closed circuit of which was connected to a free length of rubber tubing to which a lumbar puncture needle was attached. The needle was inserted into the peritoneal cavity or into a hollow viscus and air pressure was increased until disruption occurred.

Preston[42] devised an apparatus to test the effects of sutures on the strength of healing wounds. The testing apparatus consisted of a frame with parallel vertical supports holding a transverse metal rod at the top of which was suspended

a calibrated suture testing scale made by the Holland Company of Willimantic, Connecticut. Within the vertical support was placed a rectangular platform bearing a 10 kilogram weight. From the testing scale hung a system of 2 clamps which held the ends of the excised wound segment to be tested. The platform bearing the 10 kilogram weight could be hoisted and the lower tissue clamp attached to it. By lowering the platform slowly the tissue clamp system could be made taut and at this point the platform was halted and made fast by a supporting twine tied above. When the twine was cut with scissors, the falling 10 kilogram weight on the platform pulled directly against the vertically arranged tissue clamps and testing scale system. The amount of force which the incision resisted before breaking was registered on the testing scale. An accurate reading of the strength was facilitated by a ratchet which prevented the scale from snapping back when the tissue broke and the pull ceased.

Botsford[67] tested tensile strength of skin wounds by firmly clipping a Michel skin clip 3 millimeters wide or a small towel clip on each side of the wound at right angles to its approximate midpoint, and about one centimeter from it. Two hooks, one on each side, were inserted through the loops of the skin clips. The screw handle of the tensiometer was then turned slowly. This exerted constantly increasing tension upon one side of the wound whereas the other side remained fixed. The amount of tension under which the wound separated was read directly from

the spring balance. When wounds of similar size are tested in the same fashion each time, the error should be constant and the results consistent and accurate for comparison in a series.

Localio, Casale, and Hinton[68] tested wound tensile strength by inserting a 24 gauge hypodermic needle into the peritoneal cavity of the animal. It was then connected to the air bulb of a sphygmomanometer. Air was slowly pumped into the peritoneal cavity until the abdominal wall was raised from the peritoneal viscera. A second 24 gauge needle then was inserted into the peritoneal cavity. This needle was attached to a U-tube containing mercury which was calibrated in millimeters. The intraperitoneal pressure was increased slowly 2 millimeters at a time until disruption of the previously inflicted healing wound occurred.

A modification of Harvey's[26] technique was devised by Newburger[69] in 1943. He interposed a one gallon glass jar in parallel between a mercury manometer, a compressed air tank and a delivery needle. This served to equalize the rise in pressure. An intraperitoneal needle was then inserted and the animals were sacrificed under water. By sacrificing the animals under water it was possible to observe the escape of the first air bubble, which was recorded as the rupturing point of the wound.

Kobak, Benditt, Wissler, and Steffee[36] found that, by releasing air slowly from a reservoir bottle into an intra-abdominally placed condom, it was possible to measure with a

manometer the amount of pressure necessary to disrupt wounds of the abdominal wall of rats. This measurement was recorded automatically on a kymograph. After the point of separation had been determined, the pressure necessary to expand the condom through the previously disrupted incision was also established. The latter figure was considered the base line and represented the pressure dissipated in expansion of the balloon against the enclosing tissue of the peritoneal cavity and through the wound itself. By subtracting the point of separation from the base line, an objective estimate of the tensile strength of the wound could be obtained. This method tests the maximum strength of the wound, whereas in previous devices such as Harvey and Howes[27] the weakest part of the wound allowed escape of the air first.

In order to determine the tensile strength of a wound, Charney, Williamson and Bernhart[37] cut out a 0.50 centimeter strip of skin at right angles to the wound, and fastened the ends of the strip to clamps. Mercury was driven into a balloon with compressed air at a rate of 900 to 1,100 grams per minute. When the limit of strength of the skin strip was attained, it broke and the balloon in the container fell pulling out a stopper automatically halting the flow of mercury. The container with the mercury was then weighed.

A box-like rigid framework of steel, made from a standard type material commercially known as "flexframe", formed the basic structure of an apparatus devised by Williams, Mason and

Bradshaw[39] to study wound healing. Between the sides was located a plastic table adjustable in height to support an animal board. Two plastic needle-bearing type pulleys, 3 inches in diameter, were located on either side of this table and held rigidly in position. Strong nylon fish line was used to connect the metal arms attached to the abdominal wall on either side of the wound and a canvas bag below. Gradual increments of weight were added to the canvas bag by pouring into it lead shot. The tensile strength of the wound in grams was calculated as being one-half of the weight of the bag and shot. It was not an ideal apparatus from an engineering standpoint, but it was simple and gave readings with an error of only 1 to 2 per cent in the range of 0 to 5000 grams of tension.

Savlo and Dunphy[70] modified the procedure of Williams, Mason and Bradshaw[39] for the testing of tensile strength. While the abdomen of the animal was open after it was incised, two small number 32 stainless steel wire loops were placed through the abdominal wall one centimeter laterally to the wound on each side. Inside the abdomen, these loops passed through a fine polyethylene tube 8 millimeters in length, running parallel to the incision, which prevented the wires from pulling through the tissue, and distributed the pull more evenly over the length of the wound. These "pull wires" were left in place throughout the experiment. The wound was then closed with interrupted number 32 stainless steel through-and-through sutures. When the wound

was to be tested, the sutures were carefully removed and one side of the wound attached through the "pull wires" to a fixed pole. The opposite "pull wires" were attached by a fish line to a light plastic bag, suspended over a pulley. Water was flowed then at a steady rate into the plastic bag. The weight of the bag and water represented the disruption strength.

Enzinger and Warner[71] performed tensile strength tests by suspending a strip of tissue from a clamp and a light weight paper cup attached to the lower end of the strip. Water was then allowed to drop at a rate of approximately 50 milliliters per minute into the cup until the wound disrupted. The milliliters of water required to disrupt the wound was used as the index of the tensile strength.

Shafer, Beatty, and Davis[47] tested the tensile strength of wounds in a manner similar to the method described by Enzinger and Warner[71]. The procedure consisted of first removing a strip of tissue approximately 40 millimeters long and 10 millimeters wide at right angles to the original incision. One end of the tissue strip was attached to a small spring clamp, while a small paper cup was suspended from the other end of the tissue strip by another clamp. Bromoform, used because of its relatively high specific gravity, was added to the cup by a burette until the strip separated at the line of incision. Values were recorded as milliliters of bromoform required to cause separation of the wound.

Methods Of Testing Tensile Strength Of Bones.

In 1921 Allison and Brooks[72] devised a technique whereby the relative strength of bone could be determined by applying a minimal force which would break the bone when the bone was resting on two horizontal supports. The horizontal supports were constantly 25 millimeters apart, and increasing weight applied at a point mid-way between the supports was obtained by hanging a container from this point adding sand until the bone broke.

Lindsay and Howes[49] studied the tensile strength of bone by an apparatus which consisted of a standard Toledo scale provided with a support for the bone and a balanced lever arm for applying the force. The support consisted of two weight-bearing sections placed in such a relation as to form a constant span. The force was applied at two points on the bone at a fixed distance from the fracture site. With the bone resting on the supports, the force was applied at a uniform rate by allowing water to escape slowly from a reservoir into a container attached to the free end of the lever arm. The flow of water was stopped when the bone broke.

Lindsay[73] determined the breaking strength of bone by placing the bone in a horizontal position on two supports at its extremities, and gradually applying a load in the center until the bone broke. The load was applied by running water at a uniform rate of speed into a receptacle attached to the end of a lever arm resting on the bone. At the instant of

fracture, the flow of water was stopped and the amount of water in the receptacle was weighed in grams and recorded as the breaking strength.

Peyton, Anderson and Layman[74] described a somewhat different method of testing tensile strength of bone in 1934. Each end of bone was embedded in plaster of Paris. The bone was then suspended by one end, and weights were added to a pan, which was connected to the other end of the bone, until the bone broke.

Bell, Cuthburtson and Orr[53] described a technique involving bending and twisting tests for measuring the strength of bone. They embedded the ends of bones in a hard resinous cement. The apparatus used to break the bone by bending had a span produced by two parallel flat supports onto which the cast bone ends were placed. A steel wire was then looped around the center of the shaft of the bone and weights applied to a pan which was attached to the steel wire. When the bone broke, the amount of weight needed to break the bone was recorded. They measured the inside and outside diameters, as well as the thickness of the bones tested. These measurements were then applied to engineering formulae, and the breaking strength expressed in pounds per square inch.

Evans[75] stated that the breaking strength or stress of a material should be computed in terms of the load (pounds or kilograms) per unit area (square inches or square millimeters) which the material supported up to the rupture point. In order

to determine the breaking strength, Evans stated that the cross section area of the specimen of material tested must be known. Even if the cross sectional area of the bone is measured, such as Bell, Cuthburtson and Orr[53] did, the true cross sectional area of the bone cannot be computed accurately because the bone contains compact bone as well as marrow, and each varies in compactness. This is one of the many problems that prevents one from studying bone from strictly an engineering point of view.

Gardner[76] used mice and tested the tensile strength of femurs with an apparatus whereby weight was progressively and continuously added to a one centimeter span of the femur. The femur was supported on knife edged supports. Shot was passed from a hamper into a container which put pressure on one centimeter span of bone until it broke. The shot was then weighed, and this was recorded as the tensile strength. This apparatus was designed to be used with relatively light forces, with the bones breaking in a 1 to 2 kilogram range.

In 1951 Deaton, Forsyth, Coppedge and Bradshaw[58] tested the healing of fractured radii of dogs by suspending the bone on two rods. A padded lever arm, which was hinged at one end, was then placed over the middle of the bone. Weight was then added to the other end of the lever arm in 100 gram increments until the bone fractured. The amount of force applied then was determined by replacing the bone with a balance and actually measuring the force.

Savchuck[61] placed a rat bone on a knife-edged span of 14 millimeters and the knife-edged top link of a chain was centered around the bone. The chain was attached to a container below the bone, and mercury was flowed into the container at a constant rate. After the bone broke, the chain, container, and mercury were weighed. This was considered as the breaking strength of the bone.

In 1959 Savchuck[77] made a slight change in the above apparatus by adapting a commercial testing machine (Scott Testers, Inc., Providence, R. I.), to the original apparatus.

Gregory[52] studied the tensile strength of rat femurs which had been wounded, rather than fractured. He devised a special apparatus which allowed the bone to be placed on two knife-edged brackets which provided a constant span of 13.6 millimeters. A knife-edged hook was centered over the bone at mid-span which coincided reasonably well with the site of the bone wound. A container was then attached to the hook, and lead shot was added to the container at a constant flow until the bone broke. The container and shot were then weighed and this data recorded as the breaking strength.

It is apparent from this review of the numerous methods used for testing the tensile strength of bones that most workers have been dissatisfied with previous techniques and have attempted to refine these techniques for more accurate results. Unfortunately, concise reproducible methods are still lacking and must await further technical developments.

METHODS AND MATERIALS

METHODS AND MATERIALS

Soft Tissue Wound Healing Experiment.

In this portion of the study, 78 male albino rats of the Wistar strain, 26 to 27 days old were used. Their average weight at the beginning of the experiment was 53.7 grams (range 38 to 70 grams). The animals were housed 2 to a cage in wire bottom cages, and maintained at a constant temperature.

The animals were divided into a control group consisting of 34 animals, and an experimental group of 44 animals. The control animals were fed a stock laboratory diet* ad libitum and were given distilled water ad libitum. The experimental animals, fed once daily at approximately the same time, received only one porcelain cup full of pasteurized Grade A homogenized milk daily, approximately 100 milliliters. The experimental animals received no drinking water.

The animals were weighed weekly, and hemoglobin and red blood cell determinations performed on all the animals at 7th,

---

*Indiana University Stock Diet:

580# Hominy
130# Meat scraps
15# Bone meal
160# Middlings
60# Linseed meal
60# Dried buttermilk
20# Yeast culture
20# 3-way mineral
8# Alfalfa meal
1 gal. Codliver oil

21st, 34th or 35th, and 39th or 40th days after the initiation of the experiment.

The AO Spencer Bright-line Hemacytometer was used for the red blood cell determination. The acid hematin method[78] was used for the hemoglobin determination.

The blood which was used in these determinations was obtained by amputating a small section of the tip of the tail (Figure 1). It was noted that if the animals were warmed first, it was easier to obtain the necessary amount of blood. On the days that the blood determinations were carried out, the experimental animals were not fed until after the blood had been obtained. The animals, therefore, may have been slightly dehydrated, tending to give an elevated hemoglobin reading.

Due to the time element involved in operating the animals to inflict the experimental wound, they were divided into two groups. One group (18 experimentals and 14 controls) was operated 34 days after the beginning of the study. The other group (14 experimentals (Figure 3) and 12 controls (Figure 2) was operated 35 days after the beginning of the study. Anesthesia for the operation was obtained by injection of 3.5 milligrams of Abbott's Veterinary Nembutal Sodium per 100 grams of body weight. The Nembutal Sodium (60 milligrams per milliliter) was diluted with distilled water to 5 milligrams per milliliter in order to control the dosage more accurately.

After the animal had been anesthetized, blood was obtained from the tail for hemoglobin and red blood cell determinations.

Figure 1. Blood being removed from tip of rat's tail by using a Sahli pipette.

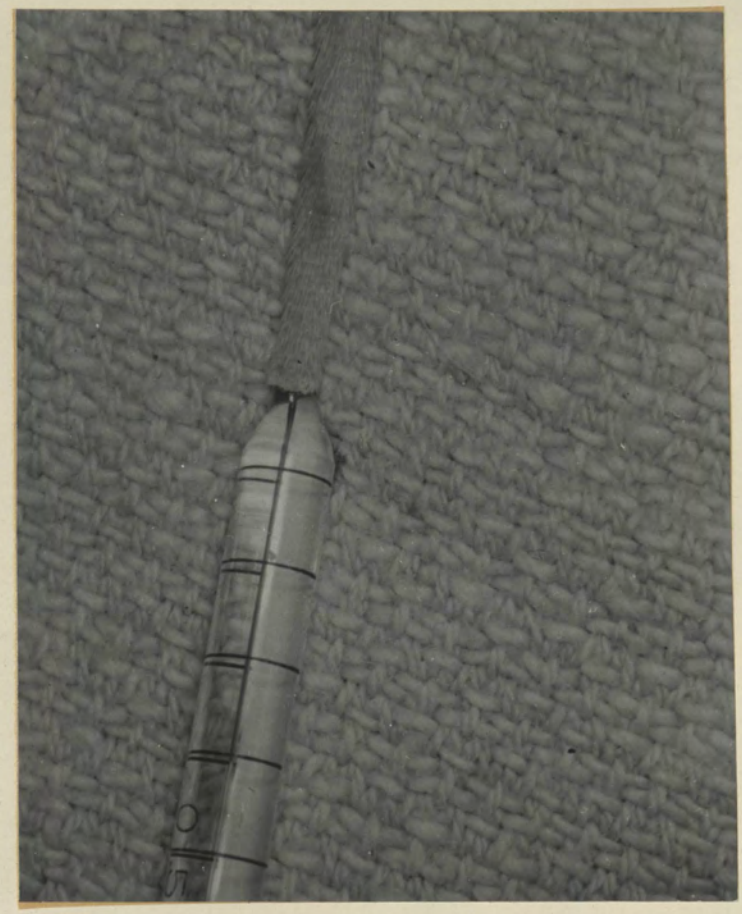


Figure 2. Clinical photograph of control animal.
Note color of eyes, nose, feet, and ears.

Figure 3. Clinical photograph of experimental animal.
Note color of eyes, nose, feet, and ears.

The hair of the abdomen of the animal then was clipped with electric hair clippers. Following this, the skin was further prepared with a depilatory (Nair) which was left on the area for about one minute; this was then thoroughly washed off, and the area was scrubbed with tincture of metaphen. In delineating the wound, a stencil made from a plastic ruler was placed on the abdomen and an indelible pencil was used to mark a standardized line for the longitudinal incision. The stencil was placed so that the incision would be 5 millimeters to the right of the midline and the anterior edge of the incision 1 centimeter below the rib cage. The stencil was used to assure an exact length and a relatively constant relationship of the wound to the animal's midline, thereby keeping all wounds in the study as nearly constant as possible.

Each incision was made with a new number 15 Bard-Parker blade through the skin down to the fascia overlying the muscle (Figure 4). The wound was then approximated with 7 evenly spaced 000 interrupted black silk sutures (Figure 5). The wound was painted with tincture of metaphen and covered with a small piece of Telfa dressing held in place with a 4 x 4 piece of gauze wrapped around the animal and taped in position. It was noted that most of the animals had removed the dressing within an hour after they recovered from the anesthesia. Otherwise, it was removed after 24 hours.

The animals tolerated the procedure well and there was only

Figure 4. Longitudinal incision in rat's abdomen made with a new number 15 Bard-Parker blade through skin down to the fascia overlying the muscle.

Figure 5. Longitudinal incision closed with 7 evenly spaced 000 interrupted black silk sutures.

one anesthetic death. Occasionally animals chewed or scratched out one or more of their sutures, but there was no gaping or gross infection of the wounds.

No attempt was made to keep the technique completely aseptic, but the blades, suture needles, and suture material were autoclaved before use. A new scalpel blade and needle were used for each animal. The other instruments were sterilized by cold sterilizing solution (Cetylcide brand of benzalkonium chloride and cetyl dimethyl ethyl ammonium bromide) before use on each animal.

Before the animals were sacrificed on the fifth postoperative day, the actual time interval being 96 ± 2 hours, blood was obtained from the tail for hemoglobin and red blood cell determinations. The animal was sacrificed then by chloroform inhalation and the wound was inspected for evidence of infection or other unusual characteristics. The remaining sutures were then carefully removed.

The tensile strength of each of the soft tissue wounds was tested by an apparatus similar to the one described by Williams, Mason, and Bradshaw[39]. The animal was placed on a platform on his back, and heavy elastic bands placed over the hind feet as well as the front feet. A stencil was placed over the wound and an indelible pencil was used to mark a point 4 millimeters laterally from the middle of the longitudinal wound. A 4 millimeter clamp was attached to the skin at one

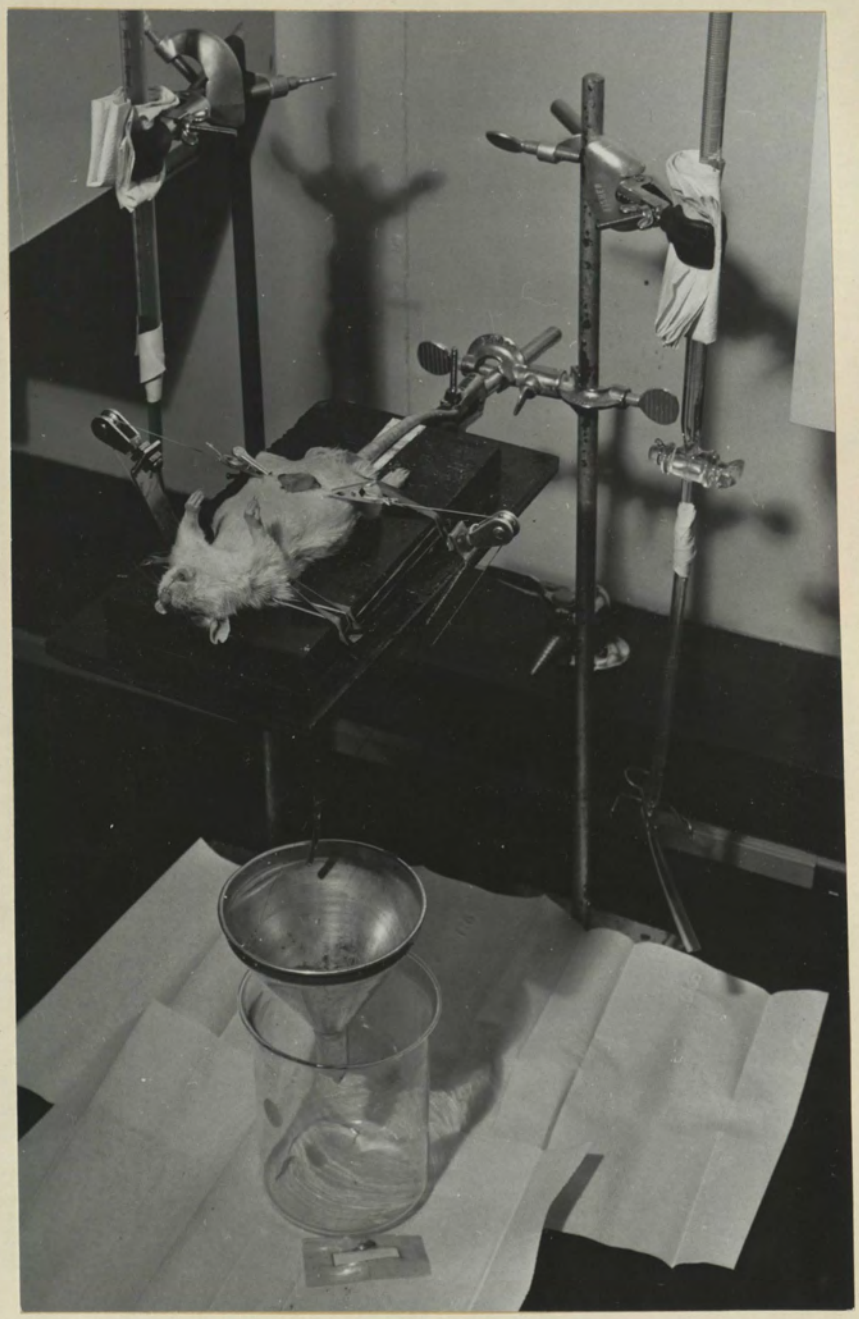
point which had been marked with the indelible pencil. This clamp was attached to a length of 18 pound test nylon fishing line which was threaded over a small pulley which in turn had a funnel attached to it. The line then was rethreaded through another pulley where another clamp was attached. This clamp was then attached to the skin at the other premarked area opposite the first clamp. The funnel was then slowly and continuously filled with bromoform from a pipette, until the wound disrupted, at which time the flow of bromoform was stopped and the reading in milliliters recorded (Figure 6).

Bone Wound Healing Experiment.

In this portion of the study, 74 male albino rats of the Wistar strain, 26 to 27 days old, were used. At the beginning of the study, their average weight was 57.6 grams (range 45 to 69 grams). The animals were housed 2 to a cage as in the previous experiment.

The animals were divided into a control group, consisting of 36 animals, and an experimental group, consisting of 38 animals. The control animals were fed the same stock laboratory diet ad libitum, and were given distilled water ad libitum. The experimental animals were fed once daily at approximately the same time. They received only one porcelain cup full of pasteurized Grade A homogenized milk daily (approximately 100 milliliters). They received no drinking water.

Figure 6. Soft tissue wound testing apparatus showing animal in place. Clamps have been placed 4 millimeters laterally from the longitudinal wound. The clamps are attached to the funnel under the animal by fishing line. Bromoform has been pipetted into the funnel thereby causing the disruption of the wound as shown.



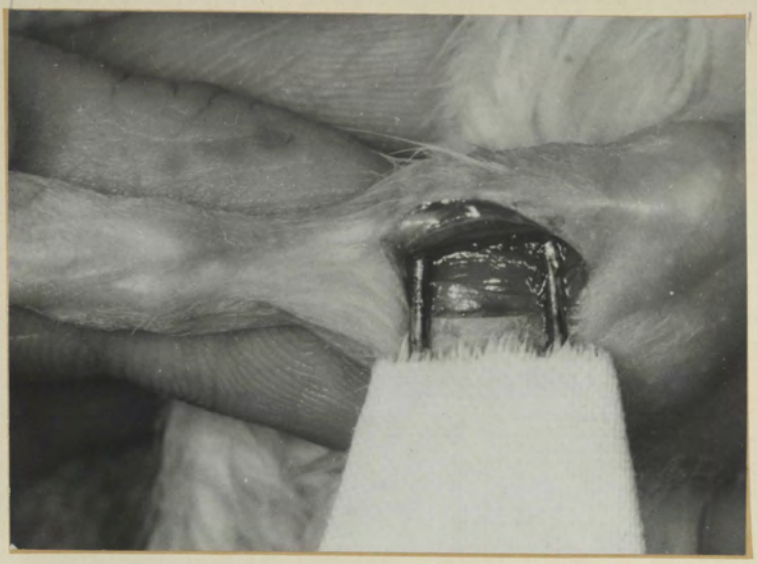
All animals were weighed weekly. Hemoglobin and red blood cell determinations were performed in the same fashion as described in the previous portion of the experiment. The blood determinations were performed on the 21st, 35th or 36th, and 42nd and 43rd days after the initiation of the study.

The animals were again divided into 2 groups, due to the time involved for the operations. The first group (22 experimentals and 22 controls) was operated 35 days after they were received while the second group (11 experimentals and 10 controls) was operated 36 days after they were received.

Preparatory to the operation, anesthesia was obtained in the same manner as in the previous soft tissue experiment. Following anesthesia, blood was obtained from the tail for hemoglobin and red blood cell determinations. The hair of the left leg and thigh was then clipped with electric hair clippers. The skin was further prepared with a depilatory (Nair) which was left on the area for about one minute. This was thoroughly removed and the area scrubbed with surgical soap followed by the application of tincture of metaphen. A longitudinal incision approximately 15 millimeters then was made along the anterior middle surface of the left tibia, and the muscle was retracted. Following this, a preformed rigid wire gauge measuring 6 millimeters from end to end, was inserted into the angle formed by the normal fusion of the tibia and fibula (Figures 7 and 8). A number 6 round

Figure 7. Placement of the wire gauge to determine the location of the bone wound.

Figure 8. Close-up view showing a dissected tibia, bone wound, and the wire gauge relationship.



steel bur next was used to drill a hole into the lateral surface of the tibia at the opposite end of the preplaced gauge. The bur was allowed to pass through the outer cortical plate and enter the marrow, but not allowed to cut into the opposite cortical plate of bone. A new bur was used on every other tibia so as to ensure sharpness and uniformity of the holes placed in the bone. The area was irrigated with a sterile solution of normal saline. A 000 black silk suture was placed in the muscle lateral to the bone wound so as to allow identification of the wound during the histologic preparation of the tissue. The skin was then approximated and closed with one metal skin clip and one 000 black silk suture (Figure 9). Tincture of metaphen was then applied to the wound and the animal returned to his cage.

The control animals tolerated the procedure better than did the experimental animals. Six experimental animals died following surgery, while there were no deaths among the control animals.

All of the animals appeared to use their wounded leg without any great difficulty immediately post-operatively and throughout the remainder of the experiment. The animals did not remove the skin clip, but in a few of the animals the skin suture was missing at the time of sacrifice.

Before the animals were sacrificed by chloroform inhalation on the seventh post-operative day, the actual healing period being 7 days $\pm$ 2 hours, blood was drawn from the tail for

Figure 9. Closure of the skin after making a bur hole in the tibia. Note skin clamp and suture.

hemoglobin determination and red blood cell count.

After each animal had been sacrificed, the wound was inspected for evidence of gross infection or other unusual characteristics. Two control and 2 experimental animals were used for histologic studies, the remainder of the animals being used for bone breaking-strength tests.

In preparation for the bone breaking-strength tests, both the right and left tibia were carefully dissected from the animal. The bones were then radiographed and subjected to the breaking strength test devised by Gregory[52].

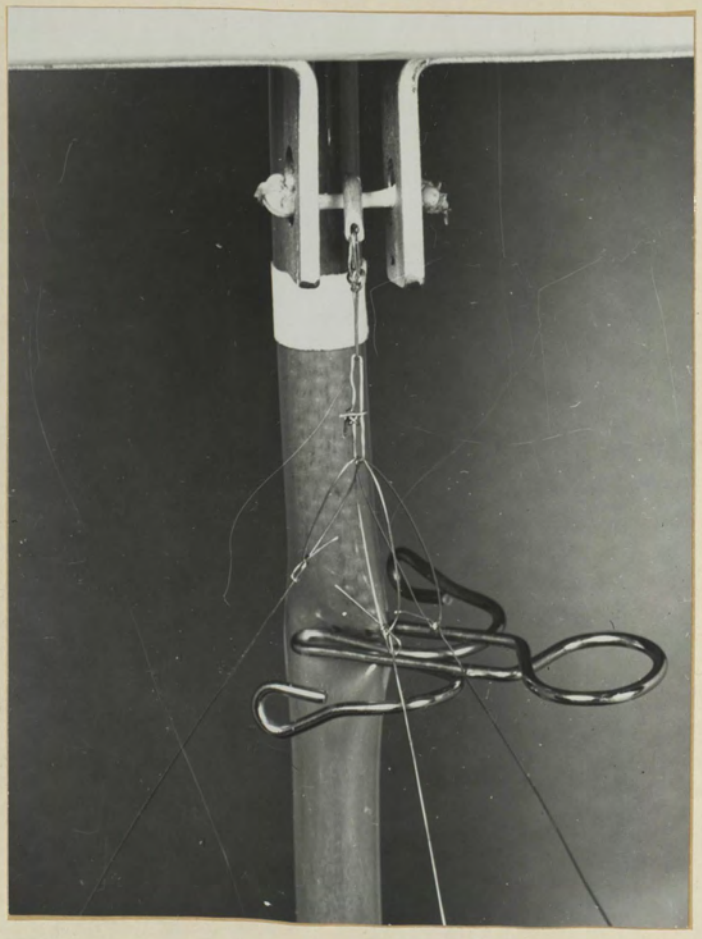
The bones were radiographed by placing both bones on an intra-oral periapical dental x-ray film which contained 2 films per packet. The film target distance was 16 inches and the exposure setting was $\frac{1}{2}$ second with the machine producing 10 milliamperes at 65 KVP. Since the film packet contained 2 films, each film was developed at different times (2 seconds and $3\frac{1}{2}$ seconds at 68°F.) to insure good contrast to the films.

The bone breaking-strength tests were performed by placing the tibia, with the wounded surface down, on 2 knife-edged brackets which provided a constant span of 13.6 millimeters. A knife-edged hook was centered over the bone at mid-span which coincided reasonably well with the site of the bone wound. A light aluminum bucket was permanently attached to the knife-edged hook by means of strong, light steel wires. Lead shot was then released by a pinchcock from a container above and

automatically poured into the aluminum bucket which was attached to the knife-edged hook. When the bone broke, the impact caused the operator to release the pinchcock and stop the flow of lead shot. The hook-wire-bucket assembly containing the lead shot was then weighed on a spring balance and the breaking strength recorded in pounds. The hook-wire-bucket assembly weighed 0.3 of a pound and the flow of lead shot was approximately $\frac{1}{2}$ pound per second (Figures 10-11).

Figure 10. Bone breaking apparatus. Large funnel at top holds the lead shot. The bucket at the bottom is attached to a knife-edged hook by way of 3 wires. The knife-edged hook is centered over the bone at mid-span. Shot is then released from the funnel into the bucket by releasing the pinchcock.

Figure 11. Close-up view of the bone breaking apparatus. The bone has been placed in the frame and the knife-edged hook with wires to the bottom bucket (not shown in this view) has been centered over the bone. The pinchcock and plastic tubing containing lead shot is shown in the background.



RESULTS

RESULTS

Soft Tissue Wound Healing Experiment.

The results of the soft tissue healing experiment are presented in Tables I and II. The tensile strength of the abdominal skin incision wounds of the 23 healed control animals was 56.0 ± 12.0 milliliters of bromoform, while for the 28 experimental animals the tensile strength was 44.2 ± 5.28 milliliters of bromoform. These data was subjected to statistical analysis* and found to be significant ($p < 0.01$).

The results of the red blood cell counts are given in Tables III and IV. The red blood cell count, before sacrifice, of 14 control animals was 5,320,000 ± 710,00 per cubic millimeter, and for the 11 other control animals carried out the following day was 5,760,000 ± 579,000 per cubic millimeter. The red blood cell count of 17 experimental animals was 2,880,000 ± 784,000 per cubic millimeter, and for the 13 other experimental animals on the following day was 2,390,000 ± 1,012,000 per cubic millimeter.

The results of the hemoglobin determinations are shown in Tables V and VI. The hemoglobin value, before sacrifice, of 14 control animals was 13.9 ± 1.3 grams per 100 millimeters of whole blood, and for the 11 other control animals on the following day was 14.4 ± 1.4 grams per 100 millimeters of whole blood.

---

* $\sigma \text{ Difference} = \sqrt{\sigma_{m_1}^2 + \sigma_{m_2}^2}$

$t = \dfrac{m_1 - m_2}{\sigma \text{ difference}}$

Table I.

TENSILE STRENGTH OF THE SOFT TISSUE CONTROL WOUNDS

Animal number	Tensile strength of wound (milliliters of bromoform)
2	31.0
3	31.0
4	46.0
5	63.0
6	59.0
7	75.0
8	69.0
9	71.0
10	71.0
12	47.0
15	48.0
16	57.5
17	54.0
18	63.0
19	73.0
21	62.0
22	68.0
23	50.0
25	49.0
31	51.0
32	49.0
33	53.0
34	49.0
Mean	56.0
Standard deviation	12.0

Table II.

TENSILE STRENGTH OF THE SOFT TISSUE EXPERIMENTAL WOUNDS

Animal number	Tensile strength of wound (milliliters of bromoform)
35	48.0
37	35.5
38	39.5
39	48.0
40	38.5
43	45.0
44	41.0
46	53.0
47	40.0
49	42.0
50	31.0
52	53.0
54	36.0
57	49.0
60	46.0
62	48.2
63	42.0
64	46.5
65	47.8
66	41.8
67	42.5
68	48.5
69	48.5
70	41.5
71	48.0
74	41.5
75	47.5
77	48.5

Mean - - - - - - - - - - - - - - - - - - 44.2
Standard deviation - - - - - - - - - - 5.3

Table III.

RED BLOOD CELL COUNT OF SOFT TISSUE EXPERIMENTAL ANIMALS

(Cells per cubic millimeters x 10^3)

Animal Number	Day of count 7	21	33	34	37	38
35	2,510	5,100	—	3,200	—	1,600
36	2,150	3,750	—	3,640	—	2,200
37	1,900	2,500	—	2,490	—	1,590
38	4,210	5,200	—	4,200	—	2,650
39	4,100	4,550	—	2,200	—	2,300
40	2,200	4,300	—	4,500	—	2,010
43	4,500	6,200	—	4,910	—	3,550
44	1,850	2,500	—	2,500	—	4,850
45	2,650	3,560	—	6,200	—	—
46	4,750	4,550	—	2,490	—	1,400
47	3,100	—	—	4,850	—	1,650
48	3,210	—	—	—	—	—
49	3,650	4,380	—	4,200	—	2,650
50	3,150	3,580	—	2,100	—	1,500
52	2,170	2,850	—	3,280	—	3,050
53	2,910	2,500	2,300	—	—	—
54	2,810	5,150	3,900	—	3,090	—
55	1,140	2,400	—	—	—	—
56	5,010	6,400	—	—	—	—
57	70	4,500	1,140	—	2,200	—
58	1,450	—	—	—	—	—
59	4,380	—	—	—	—	—
60	3,940	4,310	2,700	—	3,100	—
61	4,210	3,350	—	—	—	—
62	1,120	5,900	3,400	—	3,600	—
63	4,800	3,550	3,100	—	2,100	—
64	2,450	5,100	3,400	—	2,200	—
65	3,390	4,150	3,750	—	2,400	—
66	5,600	2,100	2,090	—	2,200	—
67	4,550	5,300	2,900	—	3,360	—
68	5,900	3,100	4,300	—	3,550	—
69	4,410	5,175	2,200	—	1,600	—
70	2,980	5,200	3,750	—	4,100	—
71	4,590	2,200	3,600	—	2,270	—
72	3,480	3,240	—	—	—	—
73	6,600	5,300	—	—	—	—
74	4,690	3,400	3,500	—	3,400	—
75	4,210	3,950	2,400	—	2,500	—
76	3,980	—	—	—	—	—
77	4,200	3,550	4,200	—	4,100	—
78	3,900	7,200	2,800	—	3,200	—
Mean	3,520	4,120	3,030	3,630	2,880	2,380
Standard Deviation					784	1,012

Table IV.

RED BLOOD CELL COUNT OF SOFT TISSUE CONTROL ANIMALS

(Cells per cubic millimeter x 10^3)

Animal Number	Day of count 7	21	33	34	37	38
1	5,200	3,500	—	4,200	—	—
2	3,620	3,100	—	6,800	—	5,750
3	2,500	3,650	—	5,210	—	6,000
4	2,240	4,350	—	4,200	—	6,100
5	5,150	3,010	—	5,200	—	4,900
6	3,500	4,900	—	6,500	—	6,070
7	5,200	3,850	—	5,600	—	5,950
8	3,010	4,100	—	6,280	—	5,010
9	4,100	6,150	—	5,950	—	5,900
10	5,100	4,810	—	5,390	—	6,400
11	5,230	4,930	—	—	—	—
12	2,520	5,390	—	6,450	—	4,800
14	3,620	4,800	—	4,100	—	6,500
15	2,520	5,100	5,450	—	4,400	—
16	4,980	5,410	3,750	—	5,090	—
17	2,510	5,090	5,200	—	5,300	—
18	2,210	4,750	4,050	—	6,100	—
19	2,830	5,100	5,100	—	5,200	—
20	4,510	6,120	5,400	—	5,100	—
21	5,230	5,380	6,400	—	4,780	—
22	1,530	3,100	5,880	—	5,950	—
23	1,620	5,820	6,800	—	—	—
24	4,530	5,310	—	—	—	—
25	5,560	3,200	5,200	—	4,800	—
26	5,100	3,100	—	—	—	—
27	3,050	5,850	—	—	—	—
30	1,120	2,550	6,100	—	6,490	—
31	1,750	4,550	6,090	—	5,200	—
32	1,300	5,050	4,600	—	4,200	—
33	3,720	2,100	5,600	—	5,900	—
34	2,630	4,050	3,500	—	6,100	—
Mean	3,540	4,410	5,270	5,490	5,320	5,760
Standard Deviation					710	597

Table V.

HEMOGLOBIN OF SOFT TISSUE EXPERIMENTAL ANIMALS

(Grams per 100 millimeters of whole blood)

Animal Number	Day of count 7	21	33	34	37	38
35	13.1	11.0	—	6.6	—	4.9
36	—	10.3	—	7.9	—	4.9
37	14.1	10.8	—	10.5	—	10.2
38	—	13.7	—	8.2	—	8.6
39	—	11.0	—	8.6	—	5.3
40	—	12.2	—	8.6	—	8.8
43	—	13.7	—	8.6	—	6.3
44	—	8.3	—	7.2	—	6.6
45	—	9.1	—	9.5	—	—
46	—	10.8	—	6.6	—	4.7
47	—	—	—	7.9	—	7.6
49	—	10.6	—	9.9	—	6.3
50	9.1	9.5	—	6.3	—	5.9
52	—	8.0	—	5.9	—	4.6
53	13.1	9.1	—	—	—	—
54	12.0	11.0	4.7	—	4.6	—
55	12.4	9.1	—	—	—	—
56	—	8.1	—	—	—	—
57	—	10.3	7.2	—	4.6	—
58	8.1	—	—	—	—	—
59	14.2	—	—	—	—	—
60	—	9.1	8.4	—	5.9	—
61	—	9.1	—	—	—	—
62	—	8.3	6.5	—	5.3	—
63	—	9.1	8.6	—	8.2	—
64	—	8.0	6.7	—	5.9	—
65	—	10.3	8.6	—	7.9	—
66	—	9.1	8.4	—	6.3	—
67	—	8.3	6.6	—	5.7	—
68	—	9.1	7.2	—	5.9	—
69	—	14.9	11.0	—	7.9	—
70	—	9.5	8.4	—	10.2	—
71	—	10.8	6.6	—	10.5	—
72	—	6.6	—	—	—	—
73	—	10.8	—	—	—	—
74	12.0	9.1	6.1	—	8.6	—
75	12.2	6.8	6.1	—	3.6	—
76	—	—	—	—	—	—
77	—	14.1	11.2	—	7.9	—
78	—	13.7	9.8	—	8.6	—
Mean	12.0	10.0	7.8	7.9	6.9	6.5
Standard Deviation					1.98	1.79

Table VI

HEMOGLOBIN OF SOFT TISSUE CONTROL ANIMALS

(Grams per 100 millimeters of whole blood)

Animal Number	Day of count 7	21	33	34	37	38
1	18.2	15.2	—	17.2	—	—
2	12.2	16.0	—	16.8	—	15.8
3	—	15.2	—	15.8	—	15.8
4	—	14.4	—	14.9	—	15.2
5	—	15.2	—	11.9	—	13.9
6	—	14.4	—	13.9	—	13.2
7	—	16.0	—	9.9	—	12.2
8	—	10.6	—	13.9	—	13.9
9	—	13.7	—	12.2	—	13.9
10	13.0	12.9	—	13.5	—	15.2
11	—	16.7	—	—	—	—
12	—	14.4	—	13.7	—	12.9
14	—	19.0	—	12.9	—	16.5
15	13.8	13.7	15.4	—	12.9	—
16	—	11.0	15.4	—	15.4	—
17	—	13.7	15.4	—	11.9	—
18	—	14.1	9.7	—	13.2	—
19	12.0	14.4	12.7	—	13.2	—
20	12.4	9.1	14.7	—	—	—
21	12.2	14.4	15.1	—	15.6	—
22	13.4	14.4	16.6	—	15.0	—
23	9.1	16.0	15.5	—	13.9	—
24	16.4	19.0	—	—	—	—
25	—	8.3	12.5	—	12.7	—
26	17.4	14.1	—	—	—	—
27	—	19.4	—	—	—	—
30	13.2	16.0	15.1	—	15.6	—
31	12.6	12.5	12.5	—	13.2	—
32	—	14.4	16.6	—	11.9	—
33	—	14.4	16.6	—	13.9	—
34	—	13.3	17.3	—	15.4	—
Mean	13.5	14.4	14.7	13.9	13.9	14.4
Standard Deviation					1.34	1.40

The hemoglobin value of 17 experimental animals was 6.9 $\pm$ 1.9 grams per 100 millimeters of whole blood, and for the 13 other experimental animals on the following day was 6.5 $\pm$ 1.7 grams per 100 millimeters of whole blood.

There was a mean hemoglobin decrease of 1.1 grams per 100 millimeters of whole blood during the 7 day healing period in the experimental animals, and a mean decrease of 0.3 grams per 100 millimeters of whole blood during the same period in the control animals.

The results of the weight gains and losses are given in Table VII. The mean weight of the control animals at the time of wounding was 189 grams, and the weight of the experimental animals was 125 grams. The mean weight of the control animals at the time of sacrifice was 208 grams, and the mean weight of the experimental animals was 128 grams.

The histologic studies did not reveal any significant differences microscopically between the control and experimental animals (Figures 12-13).

Bone Wound Healing Experiment.

The results of the bone healing experiment are presented in Tables VIII, IX, and X. The breaking strength of the bone wounds of the 27 control animals was 10.90 $\pm$ 2.52 pounds and 14.57 $\pm$ 2.18 pounds for the opposite non-wounded tibia.

The breaking strength of the bone wounds of the 25 experi-

Table VII

SOFT TISSUE STUDY

MEAN WEIGHT OF ANIMALS IN GRAMS

Days of Experiment	1	7	14	21	28	33	34	37	38
Control	55.7	68.3	90.8	131.3	169.2	201.0	183.0	220.0	192.0
Experimental	53.2	66.8	88.0	99.1	115.1	120.0	130.0	126.0	129.0

Figure 12. Photomicrograph showing good healing of a control soft tissue wound after five days healing.

Figure 13. Photomicrograph showing poor healing of a control soft tissue wound after five days healing.

Table VIII.

TENSILE STRENGTH OF THE CONTROL BONE WOUNDS

Animal Number	Tensile strength in pounds	
	Normal leg	Wounded leg
1	13.31	10.75
3	10.81	5.25
4	19.00	16.25
6	16.63	14.50
7	14.00	10.75
8	14.06	7.00
9	13.00	9.00
10	16.25	14.56
11	11.50	8.25
12	15.56	13.23
13	16.00	11.31
14	16.75	10.56
16	12.37	10.09
17	14.75	10.00
19	12.56	10.31
20	15.43	12.25
21	12.68	9.87
22	13.63	11.12
23	16.56	14.56
24	17.87	13.94
25	12.75	9.56
26	14.43	8.13
29	15.75	9.25
30	15.56	9.75
33	19.00	12.56
34	15.43	12.25
36	11.75	9.50
Mean	14.57	10.90
Standard Deviation	2.18	2.52

Table IX.

TENSILE STRENGTH OF THE EXPERIMENTAL BONE WOUNDS

Animal Number	Tensile strength in pounds	
	Normal leg	Wounded leg
37	10.25	8.75
38	10.81	7.63
39	8.63	6.00
42	9.00	6.00
43	11.75	8.00
45	10.00	7.75
46	7.81	5.81
47	8.56	4.81
48	11.56	9.12
49	12.00	9.94
50	8.00	4.12
51	9.50	6.63
52	8.50	5.50
54	10.75	7.25
56	8.00	6.50
58	8.63	6.75
59	10.25	7.50
63	9.94	7.19
64	9.50	5.56
66	7.31	5.12
67	11.56	8.56
68	9.75	3.75
69	10.43	7.00
72	8.00	6.81
73	8.50	4.25
Mean	9.56	7.45
Standard Deviation	1.36	1.81

Table X.

TENSILE STRENGTH OF UNHEALED BONES

CONTROL BONE:

Animal Number	Tensile strength in pounds Normal leg	Wounded leg	% Strength of non-wounded (left/right x 100)
5	14.81	9.25	62.5%
35	9.75	5.50	56.4%
Mean	12.28	7.37	59.4%

EXPERIMENTAL BONE:

Animal Number	Tensile strength in pounds Normal leg	Wounded leg	% Strength of non-wounded (left/right x 100)
40	8.25	4.00	48.5%
41	10.00	7.56	75.6%
53	10.31	7.50	66.3%
74	10.50	6.50	61.9%
Mean	9.76	6.38	63.0%

mental animals was 7.45 $\pm$ 1.81 pounds and 9.56 $\pm$ 1.36 pounds for the opposite non-wounded tibia.

The difference between the breaking strength of the wounded tibias in the control and experimental groups of animals was significant ($p < 0.01$). In addition, the difference in the breaking strength of the non-wounded tibia of the 2 groups was found, also, to be significant ($p < 0.01$).

The mean breaking strength of the wounded control animal bones that were tested immediately after operation was 7.37 pounds and 12.28 pounds for the non-wounded tibia. The mean breaking strength of the wounded experimental animal bones that were tested immediately after operation was 6.38 pounds and 9.76 pounds for the non-wounded tibia.

The results of the red blood cell counts are shown in Tables XI and XII. The red blood cell count before sacrifice of 21 control animals was 5,770,000 $\pm$ 801,000 per cubic millimeter and for the 9 other control animals sacrificed on the following day was 5,600,000 $\pm$ 841,000 per cubic millimeter. The red blood cell count of 18 experimental animals was 2,700,000 $\pm$ 643,000 per cubic millimeter and for the 9 other experimental animals sacrificed on the following day was 3,420,000 $\pm$ 309,000 per cubic millimeter.

The results of the hemoglobin determinations are given in Tables XIII and XIV. The hemoglobin value before sacrifice of 21 control animals was 14.80 $\pm$ 1.66 grams per 100 millimeters of

Table XI.

RED BLOOD CELL COUNT OF EXPERIMENTAL ANIMALS WITH BONE WOUNDS

(Cells per cubic millimeter x 10^3)

Animal Number	Day of count 21	34	35	41	42
37	2,600	1,800	—	2,800	—
38	3,900	3,200	—	1,500	—
39	4,380	2,200	—	2,750	—
40	4,800	4,500	—	—	—
41	4,100	2,300	—	—	—
42	3,650	1,850	—	2,400	—
43	4,100	1,750	—	2,100	—
44	3,100	2,200	—	—	—
45	3,400	3,650	—	3,600	—
46	2,800	3,850	—	2,010	—
47	3,700	3,400	—	4,200	—
48	2,500	4,400	—	2,200	—
49	4,000	4,800	—	3,100	—
50	3,400	3,750	—	2,100	—
51	3,100	2,800	—	3,200	—
52	4,400	2,670	—	3,000	—
53	2,850	3,100	—	—	—
54	3,600	3,400	—	2,970	—
55	4,090	—	—	—	—
56	2,190	2,900	—	2,800	—
58	2,990	1,980	—	2,130	—
59	2,750	2,100	—	2,090	—
60	6,100	—	—	—	—
62	3,300	4,080	—	3,700	—
63	2,300	—	5,500	—	4,300
64	5,900	—	3,100	—	5,200
65	5,900	—	3,600	—	3,200
66	3,400	—	5,600	—	3,200
67	1,950	—	3,100	—	3,600
68	4,100	—	2,800	—	2,300
69	2,500	—	4,000	—	2,000
70	3,200	—	2,600	—	—
71	1,900	—	—	—	—
72	2,600	—	2,040	—	3,600
73	5,200	—	4,900	—	3,200
74	3,100	—	2,800	—	—
Mean	3,550	3,030	3,640	2,700	3,420
Standard Deviation				643	309

Table XII.

RED BLOOD CELL COUNT OF CONTROL ANIMALS WITH BONE WOUNDS

(Cells per cubic millimeter x 10^3)

Animal Number	Day of count 21	34	35	41	42
1	5,100	5,980	—	6,100	—
2	5,900	4,800	—	4,800	—
3	6,030	4,600	—	5,100	—
4	5,090	5,800	—	6,000	—
5	4,300	3,600	—	—	—
6	4,900	5,500	—	6,400	—
7	6,090	5,400	—	5,050	—
8	7,080	4,600	—	6,060	—
9	4,350	5,100	—	6,090	—
10	3,800	6,000	—	6,800	—
11	5,900	4,500	—	4,500	—
12	5,600	5,300	—	6,600	—
13	6,000	5,000	—	6,400	—
14	3,600	6,100	—	5,100	—
16	3,900	4,800	—	6,270	—
17	6,100	6,050	—	5,000	—
19	4,800	5,100	—	5,390	—
20	5,200	6,850	—	5,010	—
21	5,200	3,650	—	7,500	—
22	3,700	6,000	—	4,800	—
23	6,100	6,070	—	5,300	—
24	4,900	5,300	—	6,900	—
25	5,400	—	5,200	—	4,700
26	5,100	—	5,300	—	6,100
27	4,300	—	6,100	—	6,600
28	5,600	—	6,800	—	4,500
29	6,000	—	5,100	—	5,710
30	5,600	—	5,000	—	6,200
31	4,850	—	—	—	—
32	6,400	—	—	—	—
33	7,900	—	6,890	—	5,600
34	5,070	—	5,090	—	5,300
35	4,400	—	5,000	—	—
36	5,010	—	5,600	—	5,690
Mean	5,270	5,280	5,600	5,770	5,600
Standard Deviation				801	841

Table XIII.

HEMOGLOBIN OF EXPERIMENTAL ANIMALS WITH BONE WOUNDS

(Grams per 100 millimeters of whole blood)

Animal Number	Day of determination 21	34	35	41	42
37	6.1	3.4	—	4.6	—
38	6.1	7.8	—	4.6	—
39	7.9	6.9	—	4.9	—
40	8.4	8.9	—	—	—
41	7.7	6.6	—	—	—
42	9.2	7.2	—	3.9	—
43	7.2	8.4	—	4.6	—
44	6.0	5.3	—	—	—
45	8.6	6.6	—	5.3	—
46	5.7	5.6	—	3.3	—
47	8.9	8.4	—	5.9	—
48	8.3	4.9	—	3.3	—
49	8.3	5.6	—	4.6	—
50	8.2	5.6	—	3.9	—
51	7.3	4.3	—	3.9	—
52	7.6	5.6	—	5.3	—
53	8.7	7.6	—	—	—
54	9.7	8.9	—	8.6	—
55	8.3	—	—	—	—
56	8.8	4.9	—	4.9	—
58	8.6	6.3	—	4.6	—
59	6.9	4.0	—	4.6	—
60	9.2	—	—	—	—
62	7.3	5.3	—	4.9	—
63	10.6	—	7.8	—	9.5
64	10.6	—	9.2	—	13.2
65	10.9	—	5.3	—	8.6
66	9.6	—	8.4	—	7.9
67	7.9	—	8.4	—	5.9
68	8.6	—	8.4	—	6.6
69	8.6	—	7.2	—	4.6
70	7.2	—	4.0	—	—
71	7.9	—	—	—	—
72	5.6	—	3.4	—	3.9
73	11.9	—	7.8	—	8.2
74	7.6	—	7.8	—	—
Mean	8.2	6.3	7.1	4.8	7.6
Standard Deviation				1.17	2.81

Table XIV.

HEMOGLOBIN OF CONTROL ANIMALS WITH BONE WOUNDS

(Grams per 100 millimeters of whole blood)

Animal Number	Day of determination 21	34	35	41	42
1	13.2	14.3	—	14.5	—
2	14.5	12.7	—	13.2	—
3	11.9	12.7	—	12.6	—
4	14.5	13.9	—	15.8	—
5	12.5	9.6	—	—	—
6	11.6	12.7	—	13.4	—
7	14.5	12.7	—	13.9	—
8	15.2	12.2	—	13.9	—
9	8.8	12.7	—	15.2	—
10	14.5	13.9	—	13.2	—
11	13.9	13.9	—	12.2	—
12	14.5	15.8	—	16.5	—
13	12.9	16.5	—	17.2	—
14	12.9	15.2	—	17.2	—
16	11.4	14.5	—	11.9	—
17	15.8	15.2	—	15.8	—
19	13.9	14.5	—	16.5	—
20	10.6	12.6	—	14.5	—
21	13.2	11.9	—	13.9	—
22	13.2	13.9	—	15.8	—
23	13.9	14.5	—	16.5	—
24	13.9	15.2	—	17.2	—
25	12.5	—	12.7	—	15.8
26	14.5	—	13.9	—	14.5
27	12.9	—	15.2	—	15.2
28	12.5	—	17.2	—	13.2
29	13.9	—	13.2	—	15.8
30	14.5	—	13.9	—	12.7
31	15.2	—	—	—	—
32	9.6	—	—	—	—
33	13.9	—	14.1	—	15.2
34	11.6	—	11.9	—	14.5
35	12.5	—	14.1	—	—
36	12.5	—	11.9	—	13.9
Mean	13.2	13.7	13.8	14.8	14.5
Standard Deviation				1.66	1.10

whole blood and for the 9 other control animals tested on the following day was 14.50 $\pm$ 1.10 grams per 100 millimeters of whole blood. The hemoglobin value of 18 experimental animals was 4.80 $\pm$ 1.17 grams per 100 millimeters of whole blood and for the 9 other experimental animals tested on the following day was 7.60 $\pm$ 2.81 grams per 100 millimeters of whole blood.

There was a mean hemoglobin decrease of 1.0 gram per 100 millimeters of whole blood during the 7 day healing period in the control animals and a mean decrease of 0.8 grams per 100 millimeters of whole blood during the same period in the experimental animals.

The results of the weight gains and losses are given in Table XV. The mean weight of the control animals at the time of wounding was 209.6 grams, while the mean weight of the experimental animals was 123.1 grams. The mean weight of the control animals at the time of sacrifice was 230.3 grams, and the mean weight of the experimental animals was 128.0 grams.

The roentgenograms of the control and experimental tibiae revealed varying degrees of radiopacity around the wounded areas, but did not show any unusual characteristics (Figures 14-15.

The histologic studies did not reveal any unusual differences microscopically between the control and experimental animals (Figures 16-17).

Table XV.

MEAN WEIGHT OF THE ANIMALS IN THE BONE STUDY IN GRAMS

	Day of experiment								
	1	7	14	21	28	34	35	41	42
Controls	60.2	82.2	107.0	146.7	170.5	211.6	205.2	225.0	242.4
Experimental	55.0	73.4	89.7	103.4	109.6	123.4	122.6	125.5	133.6

Figure 14. Roentgenograms showing typical appearance of non-wounded and wounded tibiae (note arrows) after 7 days healing in the control animals.


KS

Figure 15. Roentgenograms showing typical appearance of non-wounded and wounded tibiae (note arrows) after 7 days healing in the experimental animals.


KS


KS

Figure 16. Photomicrograph showing low magnification of the control bone wound after 7 days healing.

Figure 17. Photomicrograph showing high magnification of the control bone wound after 7 days healing.

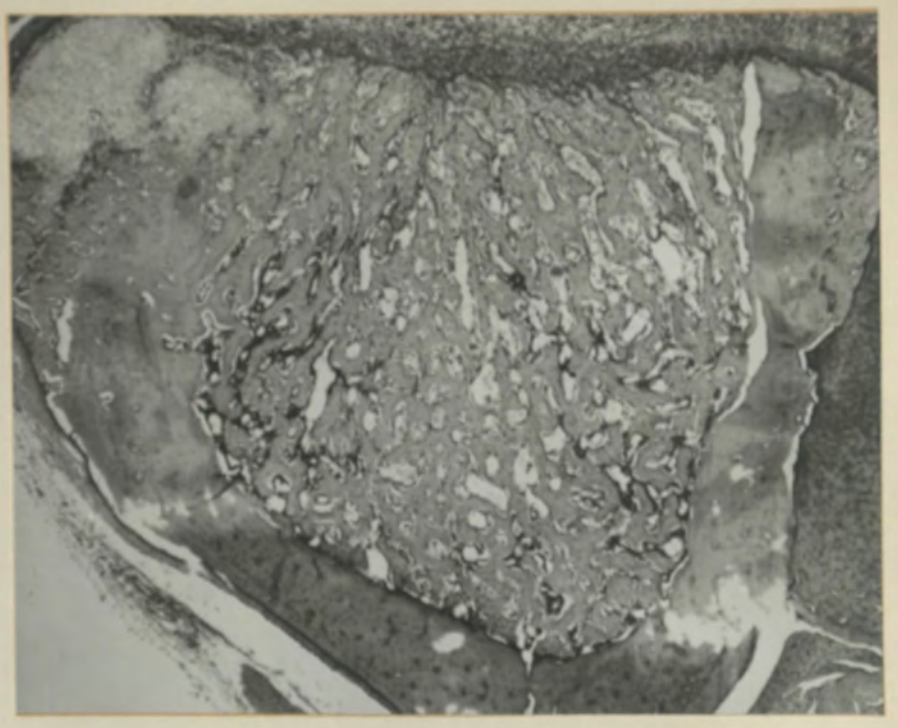
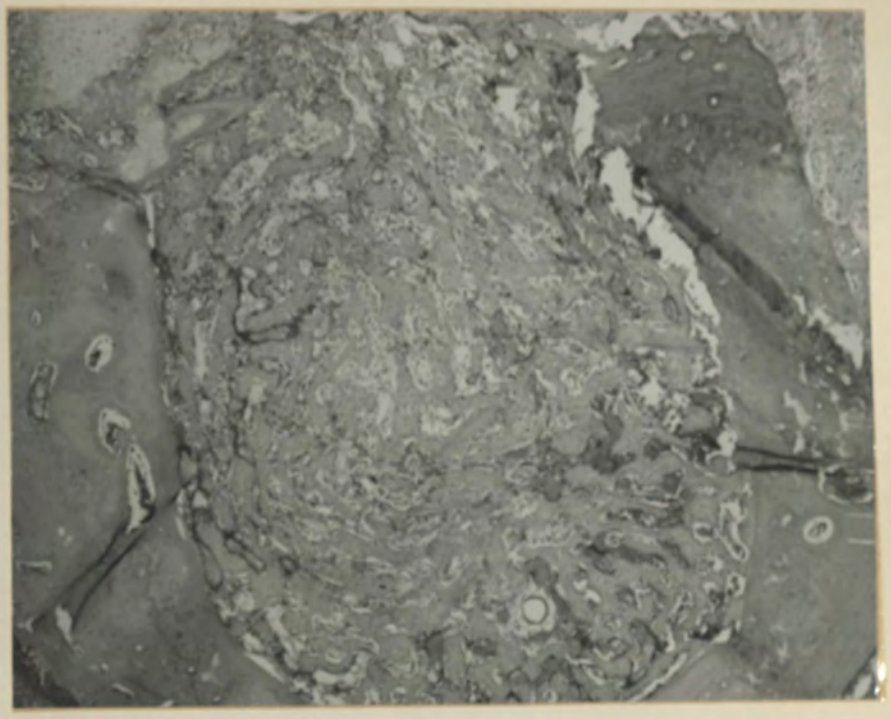


Figure 18. Photomicrograph showing low power magnification of the experimental bone wound after 7 days healing.

Figure 19. Photomicrograph showing high power magnification of the experimental bone wound after 7 days healing.



DISCUSSION

DISCUSSION

Previous experimental work concerning the effects of nutritional anemia on wound healing has not been conclusive. Besser and Ehrenhaft[22] found that acute anemia in dogs did not retard wound healing as judged by tensile strength and microscopic studies. Waterman, Burkhill, and Levenson[24] also found that nutritional anemia in albino rats did not affect the tensile strength of skin wounds. In contrast, Sandblom[23] reported that rabbits made acutely anemic had a decrease of 39.2 per cent in the tensile strength of skin wounds, while Sako[25] found, also, that acute and chronic anemia in animals resulted in a decrease in the tensile strength of healing wounds.

In a severe nutritional anemic condition there is a decrease in the number of erythrocytes and a decrease in the hemoglobin content of the blood. The primary function of erythrocytes and hemoglobin is the transportation of oxygen from the lungs to the tissues of the body. When the amount of circulating hemoglobin is reduced, there is a corresponding reduction in the oxygen-carrying capacity of the blood. The resultant anoxia of the organs and tissues of the body leads directly or indirectly to many symptoms which are common to all types of anemia.

A molecule of hemoglobin has four heme groups which are united to a protein, globin, by iron which is in the ferrous state. If inadequate iron is present in the body, insufficient heme is

formed, so there is a decrease in the hemoglobin content of the body.

It is believed that for the formation of hemoglobin, minute amounts of copper are needed. It has been stated that copper is not needed for the assimilation of iron, but it is needed for the transformation of the ingested iron into hemoglobin. A high copper content of the blood may be present, but if iron is not present the anemia will persist. The converse is true that if a high iron content is present and a deficiency of copper persists, an anemic condition will occur which closely resembles iron deficiency anemia.

When feeding young experimental animals a strict diet of milk, it is possible to induce a severe nutritional anemia, due to the lack of iron and copper in the milk. However, Cunningham[11] has stated that this anemia which develops in rats fed a diet of cow's milk may be due in part to Bartonella muris infection.

When an animal is born, it has a considerable amount of iron present for future use. During the first weeks of life there is a constant loss of iron, and almost no new iron is assimilated in the body due to the milk diet. If the diet is not altered, a nutritional anemia will ensue.

It was found as in other experimental work that a nutritional anemia could be produced by feeding only milk to the experimental animals. It was felt that the determinations of red blood cell counts and hemoglobin determinations were adequate

tests for the judgement of the severity of the anemic state of the animals. These tests were performed without difficulty or without alteration of the blood volume of the animals. A serum protein determination would have been helpful to determine the protein state of the animal, but it was not performed since it was not considered particularly germane.

Histological studies were performed to determine if there were any obvious differences in the healing wounds of the anemic and non-anemic animals. Inasmuch as the experimental study was primarily mechanical in nature, only a few sections of each representative group were made. No unusual differences were noted between the soft tissue sections or the bone sections of the anemic and non-anemic animals.

The radiographs of the bone wounds were not significant with respect to differences between the anemic and non-anemic group of animals. A slight difference was noted in the over-all size of the bone, with the non-anemic animals having the larger bones. This increase in size was expected due to the differences in weight.

The apparatuses used to test the bone and soft tissue wounds were not mechanically ideal for testing of wounds, but they are basically sound and very functional. Although problems of testing healing wounds leave much to be desired as far as finding a flawless technique, the techniques used in this experiment were kept as constant and standardized as possible.

The significant results which resulted from this experimental work may have been influenced by the great differences in the weights of the animals. The difference in weight, at the time of sacrifice, of the control and experimental soft tissue animals was 102.3 grams. The weight difference in the bone study was 80.0 grams.

A control study should be conducted to test the healing strengths of soft tissue and bone wounds in control animals weighing the same as the experimental anemic animals. This type of study would be beneficial in determining the effect of the difference in weight between the control and experimental animals.

SUMMARY

SUMMARY

A review of the literature revealed that the findings concerning the effects of nutritional anemia on experimental wound healing was not conclusive. Therefore, an experiment was performed to determine if a nutritional anemia affected wound healing of the skin and bone of rats. Mechanical methods of testing the wounds were used primarily, with histological and roentgenographic methods as supplements.

In the soft tissue experiment, 78 male rats of the Wistar strain were divided into 2 groups and the experimental group received only milk as the daily feeding. The anemic condition of the animals was measured by hemoglobin and red blood cell counts. At the end of 5 weeks the animals were subjected to a surgical operation wherein a uniform skin incision was made in the abdominal area. Following a 5 day healing period the surviving animals were sacrificed and the wounds tested on a tensile strength testing apparatus.

Histological examinations were made on representative control and experimental animals. The histological examinations did not reveal any significant differences between the two groups. There was a significant difference ($p < 0.01$) between the tensile strength of the healing skin wounds of the control and experimental animals, which suggests that, under the conditions of the experiment, there is a relationship between the tensile strength of healing skin

wounds and anemia. The great differences in the weight of the control and the experimental animals may be an important influencing factor, however.

In the bone experiment, 74 male rats of the Wistar strain were divided into 2 groups and the experimental group received only milk as the daily diet. The systemic anemic condition of the animals was determined by hemoglobin and red blood cell counts. At the end of 5 weeks the animals were subjected to a surgical operation wherein a uniform bur hole was placed in the tibia. Following a 7 day healing period, the surviving animals were sacrificed, the tibiae were dissected free of all soft tissue and radiographed, and the wounds were tested on a tensile strength testing apparatus.

Histological examinations were made of representative control and experimental animals, but no differences were noted between the two groups. No difference could be detected in the degree of healing between the control and experimental groups radiographically. The radiographic study did show that the control group had larger tibiae. There was a significant difference ($p:<0.01$) between the tensile strength of the control and experimental healing bone wounds, which suggests that under the conditions of the experiment there is a relationship between the tensile strength of experimental healing bone wounds and anemia. The great difference in the weight and size of the tibia may be an important influencing factor.

CONCLUSIONS

CONCLUSIONS

1. It is possible to produce severe nutritional anemia in rats by placing them on a pure milk diet for 4 to 6 weeks.
2. It was noted that the tensile strength of the skin wounds of the anemic rats was significantly less than that of the non-anemic control rats.
3. It was noted that the tensile strength of the bone wounds of the anemic rats was significantly less than that of the non-anemic control rats.
4. The difference in weight of the control and experimental soft tissue animals, at the time of sacrifice, was 102.3 grams. The weight difference in the bone study was 80.0 grams. A control study should be performed to determine if this variation in weight of the control and experimental animals affects the tensile strength of the soft tissue and bone wounds.

REFERENCES

REFERENCES

1. Mason, M. L.: Wound healing. Internat. Abstr. Surg., 69:312, 1939.

2. Whipple, A. O.: The critical latent or lag period in the healing of wounds. Ann. Surg., 112:481, 1940.

3. Beutler, E.: Iron content of haemoglobin in iron deficiency. Nature, London, 181:837, 1958.

4. Fowler, W. M.: Hematology for students and practitioners. Paul Hoeber, Inc., New York, p. 124, 1949.

5. Schwartz, S. O., and Flowers, V. C.: Morphologic changes in red blood cell with iron deficiency anemia. J.A.M.A., 130:622, 1946.

6. Mitchell, H. S., and Schmidt, L.: The relation of iron from various sources to nutritional anemia. J. Biol. Chem., 70:471, 1926.

7. Waddell, J., Steenbock, H., Elvehjem, C. A., and Hart, E. B.: Iron in nutrition. J. Biol. Chem., 77:769, 1928.

8. Elvehjem, C. A., and Kemmerer, A. R.: Improved technique for production of nutritional anemia in rats. J. Biol. Chem., 93:189, 1931.

9. Robscheit-Robbins, F. S., and Whipple, G. H.: Hemoglobin production increases with severity of anemia. Am. J. Physiol., 134:263, 1941.

10. Smith, S. E., and Medlicott, M.: Blood picture of iron and copper deficiency anemia in the rat. Am. J. Physiol., 141: 354, 1944.

11. Cunningham, I. J.: Some biochemical and physiological aspects of copper in animal nutrition. Biochem. J., 25:1267, 1931.

12. Gorter, E.: Copper and anemia. Amer. J. Dis. Child., 46: 1066, 1933.

13. Elvehjem, G. A.: The biological significance of copper and its relation to iron metabolism. Physiol. Rev., 15:471, 1935.

14. Schultze, M. O., Elvehjem, C. A., and Hart, E. B.: Studies on the copper content of the blood in nutritional anemia. J. Biol. Chem., 116:107, 1936.

15. Symposium on copper metabolism. Johns Hopkins University, Baltimore, Maryland, p. 261, 1950.

16. Teague, H. S., and Carpenter, L. E.: The demonstration of copper deficiency in growing pigs. J. Nutrit., 43:389, 1951.

17. Lahey, M. E., Gubler, C. J., Chase, M. S., Cartwright, G. E., and Wintrobe, M. M.: Studies on copper metabolism. II. Hematologic manifestations of copper deficiency in swine. Blood 7:1053, 1952.

18. Baxter, J. H., Van Wyk, J. J., and Follis, R. H., Jr.: A bone disorder associated with copper deficiency. II. Histological and chemical studies on bones. Johns Hopkins Hosp. Bull., 93:25, 1953.

19. Baxter, J. H., and Van Wyk, J. J.: A bone disorder associated with copper deficiency. I. Gross morphological, roentgenological and chemical observations. Johns Hopkins Bull., 93:1, 1953.

20. Van Wyk, J. J., Baxter, J. H., Akeroyd, J. H., and Motulsky, A. G.: The anemia of copper deficiency in dogs compared with that produced by iron deficiency. Johns Hopkins Hosp. Bull., 93:41, 1953.

21. Follis, R. H., Jr., Bush, J. A., Cartwright, G. H., and Wintrobe, M. M.: Johns Hopkins Hosp. Bull., 97:405, 1955.

22. Besser, E. L., and Ehrenhaft, J. L.: The relationship of acute anemia to wound healing. Surg., 14:239, 1943.

23. Sandblom, Philip: The tensile strength of healing wounds. Acta. Chir. Scandinav., 90:1, 1948.

24. Waterman, D. F., Birkhill, F. R., and Levenson, S. M.: The healing of wounds in the presence of anemia. Medical Nutrition Laboratory U. S. Army Report. 88, Oct., 1951.

25. Sako, Y., Kremen, A., and Varco, R. L.: Measurements of healing strength of a standard wound in anemic or starvation states and after treatment with blood transfusions or refeeding. Am. College of Surgeons, Bulletin. 34:43, 1949.

26. Harvey, S. C.: Velocity of growth of fibroblasts in healing wounds. Arch. Surg., 18:1227, 1929.

27. Harvey, S. C., and Howes, E. L.: Effect of high protein diet on the velocity of the growth of fibroblasts in healing wounds. Ann. Surg., 91:641, 1930.

28. Howes, E. L., and Harvey, S. C.: The age factor in the velocity of the growth of fibroblasts in the healing wound. J. Exp. Med., 55:577, 1932.

29. Howes, E. L., Briggs, H., Shea, R., and Harvey, S. C.: The effect of complete and partial starvation on the rate of fibroplasia in the healing wound. Arch. Surg., 27:846, 1933.

30. Howes, E. L., and Harvey, S. C.: Clinical significance of experimental studies in wound healing. Ann. Surg., 102: 941, 1935.

31. Smelo, L. S.: Problem of wound healing; effects of local agents. Arch. Surg., 33:493, 1936.

32. Lanman, T. H., and Ingalls, T. H.: Vitamin C deficiency and wound healing. Ann. Surg., 105:616, 1937.

33. Taffel, M., and Harvey, S. C.: Effect of absolute and partial vitamin C deficiency on healing wounds. Proc. Soc. Exper. Biol. and Med., 38:518, 1938.

34. Burr, H. S., Taffel, M., and Harvey, S. C.: An electrometric study of the healing wound in man. Yale J. Biol., 12:483, 1940.

35. Thompson, W. D., Ravdin, I. S., Rhoads, J. E., and Frank, I.: Effect of hypoproteinemia on wound disruption. Arch. Surg., 36:500, 1938.

36. Kobak, M. W., Benditt, E. P., Wissler, R. W., and Steffee, C. H.: The relation of protein deficiency to experimental wound healing. Surg. Gynec. and Obst., 85:751, 1947.

37. Charney, J., Williamson, M. B., and Bernhart, F. W.: An apparatus for the determination of the tensile strength of healing wounds. Science, 105:396, 1947.

38. Fendlay, C. W., Jr., and Howes, E. L.: Effect of edema on tensile strength of incised wounds. Surg., Gynec., and Obst., 90:670, 1950.

39. Williams, R. W., Mason, L. B., and Bradshaw, H. H.: Factors affecting healing. S. Forum. p. 410, 1951.

40. Lawrence, J. S., Pearse, H. E., and Mider, G. B.: Effect of experimental neutropenia on healing. Arch. Path., 28:32, 1939.

41. Burr, H. S., Harvey, S. C., and Taffel, M.: Bioelectric correlates of wound healing. Yale J. Biol. and Med., 11: 103, 1938.

42. Preston, D. J.: The effects of sutures on the strength of healing wounds. Am. J. Surg., 49:56, 1940.

43. Localio, A. S., Casafe, W., and Hinton, J. W.: Wound healing - experimental and statistical study. Surg., Gynec., and Obst., 77:81, 1943.

44. Chassin, J. L., McDougall, H. A., MacKay, M., and Localio, S. A.: Effect of stress upon the healing of wounds in rats. Proc. Soc. Exper. Biol. and Med., 83:798, 1953.

45. Savlo, E. D., and Dunphy, J. E.: Healing of disrupted and resutured wounds. Surg., 36:362, 1954.

46. Borgstrom, S., and Sandblom, P.: Suture technique and wound healing. Ann. Surg., 144:982, 1956.

47. Shafer, W. G., Beatty, R. E., and Davis, W. B.: Effect of dilantin sodium on the tensile strength of healing wounds. Proc. Soc. Exp. Biol. and Med., 98:348, 1958.

48. Nishihara, G., and Prudden, J. F.: A quantitative relationship of wound tensile strength to length. Surg., Gynec., and Obst., 107:305, 1958.

49. Lindsay, M. K., and Howes, E. L.: The breaking strength of healing fractures. J. Bone and Joint Surg., 13:491, 1931.

50. McKeown, R. M., Lindsay, M. K., Harvey, S. C., and Howes, E. L.: The breaking strength of healing fractured fibulae of rats. A. M. A. Arch. Surg., 24:458, 1932.

51. Stuck, W. G.: The effect of insulin on the healing of experimental fractures in the rabbit. J. Bone and Joint Surg., 14: 109, 1932.

52. Gregory, W. B.: The effect of insulin on the healing of bone wounds and soft tissue wounds in albino rats. Thesis, Indiana University School of Dentistry. p. 60, 1959.

53. Bell, G. H., Cuthburtson, D. P., and Orr, J.: Strength and size of bone in relation to calcium intake. J. Physiol., 100:299, 1941.

54. Bell, G. H., Chambers, J. W., and Dawson, I. M.: Mechanical and structural properties of bone in rats on rachitogenic diet. J. Physiol., 106:286, 1947.

55. Bourne, G. H.: Some experiments on the possible relationship between vitamin C and calcification. J. Physiol., 102:319, 1942.

56. Bell, G. H., and Cuthbertson, D. P.: Effect of various hormones on chemical and physical properties of bone. J. Endocrinol., 3:302, 1943.

57. Gardner, W. U.: Influence of sex and sex hormones on the breaking strength of bones of mice. Endocrinology. 32:149, 1943.

58. Deaton, W. R., Jr., Forsyth, F., Coppedge, T. O. and Bradshaw, H. H.: The sex hormones in fracture healing: an experimental study. Surg., Gynec., and Obst., 93:763, 1951.

59. Eskelund, V., and Plum, C. M.: Experimental investigations into the healing of fractures - influence of extracts of thymus and other tissues. Acta. Endocrinol., 12:171, 1953.

60. Gillespie, J. A.: The nature of the bone changes associated with nerve injuries and disuse. J. Bone and Joint Surg., 36 B: 464, 1954.

61. Savchuck, W. B.: Effects of strontium and fluorine on the repair of unreduced humeral fractures in the adult rat. J. Bone and Joint Surg., 39 A: 140, 1957.

62. Fahim, M. S. A.: Histologic study of bone repair in lathyritic albino rats. Thesis, Indiana University School of Dentistry. p. 43, 1958.

63. Howes, E. L., Sooy, J., and Harvey, S. C.: The healing of wounds as determined by their tensile strength. J. A. M. A., 92:42, 1929.

64. Ellis, J. D.: The rate of healing of electro-surgical wounds as expressed by tensile strength. J. A. M. A., 96:16, 1931.

65. Burr, H. S., and Hovland, C. I.: Bio-electric potential gradients in the chick. Yale J. Biol. and Med., 9:247, 1937.

66. Burr, H. S., Harvey, S. C., and Taffel, M.: Bio-electric correlates of wound healing. Yale J. Biol., 11:103, 1938.

67. Botsford, T. W.: The tensile strength of sutured skin wounds during healing. Surg., Gynec., and Obst., 72:690, 1941.

68. Localio, S. A., Cassle, W., and Hinton, J. W.: Wound healing experimental and statistical. III. Experimental observations. Surg., Gynec., and Obst., 77:244, 1943.

69. Newburger, B.: Early postoperative walking. I. The influence of exercise on wound healing in rats. Surg., 13:692, 1943.

70. Savlov, E. D., and Dunphy, J. E.: Mechanisms of wound healing; a comparison of preliminary local and distant incisions. New England J. Med., 250:1062, 1954.

71. Enzinger, F. M., and Warner, E. D.: Wound healing in experimental lathyrism. Lab. Invest., 6:251, 1957.

72. Allison, N., and Brooks, B.: Bone atrophy, an experimental study of the changes in bone which result from non-use. Surg., Gynec., and Obst., 33:250, 1921.

73. Lindsay, M. K.: Observations on fracture healing in rats. J. Bone and Joint Surg., 16:162, 1934.

74. Peyton, W. T., Anderson, U. S., and Layman, C. W.: Quantitative study of the rate of healing in bone. I. Description of method. A. M. A. Arch. Surg., 29:23, 1934.

75. Evans, F. G.: Stress and strain in bones. Charles C. Thomas, Publisher, Springfield, Illinois, Ch. 13, p. 162, 1957.

76. Gardner, W. U.: Influence of sex and sex hormones on breaking strength of bones of mice. Endocrinology. 32:149, 1943.

77. Savchuck, W. B.: A comparison of bone growth in normal and strontium treated rats. J. D. Res., 38:49, 1959.

78. Klett-Summerson Photoelectric Colorimeter Clinical Manual. Klett Manufacturing Co., New York, July, 1954.

BIBLIOGRAPHY

BIBLIOGRAPHY

Allison, N., and Brooks, B.: Bone atrophy. An experimental study of the changes in bone which result from non-use. Surg., Gynec., and Obst., 33:250, 1921.

Amprino, R.: Investigation on some physical properties of bone tissue. Acta. Anat. Basel., 34:161, 1958.

Arey, L.: Physiol. Rev., 16:327, 1936.

Babcock, W. W.: Wounds and their complications. Am. J. Surg., 36:3, 1937.

Barer, A. P., and Fowler, W. M.: A comparison of the hemoglobin response to varying dosages of iron. J. Lab. and Clin. Med., 26:1482, 1941.

Baxter, J. H., and Van Wyk, J. J.: A bone disorder associated with copper deficiency. I. Gross morphological, roentgenological and chemical observations. Johns Hopkins Bull., 93:1, 1953.

Baxter, J. H., Van Wyk, J. J., and Follis, R. H., Jr.: A bone disorder associated with copper deficiency. II. Histological and chemical studies on bones. Johns Hopkins Hosp. Bull., 93:25, 1953.

Bell, G. H.: Remarks on the growth and healing of bone. Brit. M. Bull., 31:76, 1945.

Bell, G. H., Chambers, J. W., and Dawson, I. M.: Mechanical and structural properties of bone in rats on rachitogenic diet. J. Physiol., 106:286, 1947.

Bell, G. H., Chambers, J. W., and Waddell, M. B.: The routine estimation of hemoglobin as oxyhemoglobin. Biochem. J. 39:60, 1945.

Bell, G. H., and Cuthbertson, D. P.: Effect of various hormones on chemical and physical properties of bone. J. Endocrinol., 3:302, 1943.

Bell, G. H., Cuthbertson, D. P., and Orr, J.: Strength and size of bone in relation to calcium intake. J. Physiol. 100: 299, 1941.

Besser, E. L., and Ehrenhaft, J. L.: The relationship of acute anemia to wound healing. Surg., 14:239, 1943.

Beutler, E.: Iron content of haemoglobin in iron deficiency. Nature, London, 181:837, 1958.

Beutler, E., Robson, M. J., and Buttenwieser, E.: A comparison of the plasma iron, iron binding capacity of sternal marrow iron, and other methods in the clinical evaluation of iron stores. Ann. Int. M., 48:60, 1958.

Bloom, W.: Resorption of bone, metabolic interrelations. Trans. Josiah Macy, Jr., Found., 3:11, 1951.

Borgstrom, S., and Sandblom, P.: Suture technique and wound healing. Ann. Surg., 144:982, 1956.

Botsford, T. W.: The tensile strength of sutured skin wounds during healing. Surg., Gynec., and Obst., 72:690, 1941.

Bourne, G. H.: Some experiments on the possible relationship between vitamin C and calcification. J. Physiol., 102: 319, 1942.

Burr, H. S., Harvey, S. C., and Taffel, M.: Bio-electric correlates of wound healing. Yale J. Biol., 11:103, 1938.

Burr, H. S., and Hovland, C. I.: Bio-electric potential gradients in the chick. Yale J. Biol. and Med., 9:247, 1937.

Burr, H. S., Taffel, M., and Harvey, S. C.: An electrometric study of the healing wound in man. Yale J. Biol., 12:483, 1940.

Caffey, J.: Pediatric X-ray diagnosis. Ed. 3, Chicago, Yearbook, 1956.

Caffey, J.: Skeletal changes in chronic hemolytic anemias, (erythroblastic anemia, sickle cell anemia and chronic hemolytic icterus), Am. J. Roentgeno., 37:293, 1937.

Carrel, Alexis: The treatment of wounds. J. A. M. A., 55:2148, 1910.

Carrel, A., and Hartmann, A.: Cicatrization of Wounds, the relation between the size of a wound and the rate of its cicatrization. J. Exp. M., 24:429, 1916.

Carrel, A., and du Nouy, P. L.: Cicatrization of wounds: latent period. J. Exp. M., 34:339, 1921.

Cartwright, G. E., Lauritsen, M. A., Humphreys, S., Jones, P. J., Merrill, I. M., and Wintrobe, M. M.: The anemia of infection. II. The experimental production of hypoferremia and anemia in dogs. J. Clin. Invest., 25:81, 1946.

Charney, J., Williamson, M. B., and Bernhart, F. W.: An apparatus for the determination of the tensile strength of healing wounds. Science. 105:396, 1947.

Chassin, J. L., McDougall, H. A., MacKay, M., and Localio, S. A.: Effect of stress upon the healing of wounds in rats. Proc. Soc. Exper. Biol. and Med., 83:798, 1953.

Chlumsky, F.: Ueber verschiedene methoden der darmvereinigung. Bruns. Beitr., 25:539, 1899.

Clark, A. H.: The effect of diet on the healing of wounds. Bull., Johns Hopkins Hospital, 30:117, 1919.

Cooley, T. B., and Lee, P.: Series of cases of splenomegaly in children with anemia and peculiar bone change. Trans. Am. Pediat. Soc., 37:29, 1925.

Cunningham, I. J.: Some biochemical and physiological aspects of copper in animal nutrition. Biochem. J., 25:1267, 1931.

Curry, J. D.: Differences in the tensile strength of bone of different histological types. Jour. of Anat., London, 93:87, 1959.

Davidson, J. N.: Humoral aspects of healing. Brit. M. Bull., 3:73, 1945.

Deaton, W. R., Jr., Forsyth, F., Coppedge, T. O., and Bradshaw, H. H.: The sex hormones in fracture healing: an experimental study. Surg., Gynec., and Obst., 93:763, 1951.

Donaldson, H. H.: The rat. Philadelphia, 2nd Ed., 1925.

Du Nouy, P. L.: Cicatrization of wounds. II. Mathematical expression of the curve representing cicatrization. J. Exper. Med., 24:451, 1916.

Ebling, A. H.: Cicatrization of wounds: temp. coefficient. J. Exper. Med., 35:657, 1922.

Edwards, L. C., and Dunphy, J. E.: Wound healing. II. Injury and abnormal repair. New England J. Med., 259:275, 1958.

Ellis, J. D.: The rate of healing of electro-surgical wounds as expressed by tensile strength. J. A. M. A., 96:16, 1931.

Elvehjem, C. A.: The biological significance of copper and its relation to iron metabolism. Physiol. Rev., 15:471, 1935.

Elvehjem, C. A., and Kemmerer, A. R.: Improved technique for production of nutritional anemia in rats. J. Biol. Chem., 93:189, 1931.

Enzinger, F. M., and Warner, E. D.: Wound healing in experimental lathyrism. Lab. Invest., 6:251, 1957.

Eskelund, V., and Plum, C. M.: Experimental investigations into the healing of fractures - influence of extracts of thymus and other tissues. Acta. Endocrinol., 12: 171, 1953.

Eskelund, V., and Plum, C. M.: Experimental investigations into the healing of fractures. Acta. Orthop. Sand., 19:433, 1950.

Evans, F. G.: Stress and strain in bones. Charles C. Thomas, Publisher, Springfield, Illinois, Ch. 13, p. 162, 1957.

Evans, F. Gaynor, Pedersen, H. E., and Lissner, H. R.: The role of T. S. in the mechanism of femoral fractures. J. Bone and Joint Surg., 33 A: 485, 1951.

Fahim, M. S. A.: Histologic study of bone repair in lathyritic albino rats. Thesis, Indiana University School of Dentistry, p. 43, 1958.

Fendlay, C. W., Jr., and Howes, E. L.: Effect of edema on tensile strength of incised wounds. Surg., Gynec., and Obst., 90:670, 1950.

Follis, R. H., Jr., Bush, J. A., Cartwright, G. H., and Wintrobe, M. M.: Johns Hopkins Hosp. Bull., 97:405, 1955.

Fowler, W. M.: Hematology for students and practitioners. Paul Hoeber, Inc., New York, p. 124, 1949.

Gardner, W. U.: Influence of sex and sex hormones on the breaking strength of bones of mice. Endocrinology. 32:149, 1943.

Gaynor, E. F., and Lebow, M.: The strength of human compact bone as revealed by engineering technics. Amer. J. Surg., 83:326, 1952.

Gillespie, J. A.: The nature of the bone changes associated with nerve injuries and disuse. J. Bone and Joint Surg., 36 B: 464, 1954.

Gorter, E.: Copper and anemia. Amer. J. Dis. Child., 46:1066, 1933.

Grace, R. V.: Disruption of abdominal wounds. Ann. Surg., 99: 28, 1934.

Gregory, W. B.: The effect of insulin on the healing of bone wounds and soft tissue wounds in albino rats. Thesis, Indiana University School of Dentistry. p. 60, 1959.

Greig, W. A.: The effects of additions of calcium carbonate to the diet of breeding mice. Brit. J. Nutrit., 6:280, 1952.

Hagedorn, A. B.: The diagnosis and treatment of iron deficiency anemia. Med. Clin. N. America, 40:983, 1956.

Ham, T. H., Castle, W. B., Gardner, F. H., and Daland, G. A.: Laboratory diagnosis of ploycythemia and anemia. N. England J. M., 243:22, 1950.

Harris, L. J.: Conference on wound healing. Bull. War. Med., 4:251, 1944.

Harvey, S. C.: The healing of the wound as a biologic phenomenon. Surg., 25:655, 1949.

Harvey, S. C.: Velocity of growth of fibroblasts in healing wounds. Arch. Surg., 18:1227, 1929.

Harvey, S. C., and Howes, E. L.: Effect of high protein diet on the velocity of the growth of fibroblasts in healing wounds. Ann. Surg., 91:641, 1930.

Heath, C. W., and Patek, A. J.: The anemia of iron deficiency. Medicine. 16:267, 1937.

Howes, E. L.: The relationship of the general condition of the patient to the healing of the open wound. Internat. Abst. Surg., 68:550, 1939.

Howes, E. L., Briggs, H., Shea, R., and Harvey, S. C.: The effect of complete and partial starvation on the rate of fibroplasia in the healing wound. Arch. Surg., 27:846, 1933.

Howes, E. L., and Harvey, S. C.: The strength of the healing wound in relation to the holding strength of catgut suture. New England J. Med., 200:85, 1929.

Howes, E. L., and Harvey, S. C.: Clinical significance of experimental studies in wound healing. Ann. Surg., 102:941, 1935.

Howes, E. L., and Harvey, S. C.: The age factor in the velocity of the growth of fibroblasts in the healing wound. J. Exp. Med., 55:577, 1932.

Howes, E. L., Sooy, J., and Harvey, S. C.: The healing of wounds as determined by their tensile strength. J. A. M. A., 92:42, 1929.

Hudack, S. S., and Blunt, J. W., Jr.: Basic processes of healing. Am. J. Surg., 80:680, 1950.

Jenkins, H. P.: A clinical study of catgut in relation to abdominal wound disruption. Surg., Gynec., and Obst., 64:648, 1937.

Josephs, H. W.: Iron metabolism in infancy. Bull., Johns Hopkins Hosp., 55:259, 1934.

Klett-Summerson Photoelectric Colorimeter Clinical Manual. Klett Manufacturing Co., New York, 1954.

Kobak, M. W., Benditt, E. P., Wissler, R. W., and Steffee, C. H.: The relation of protein deficiency to experimental wound healing. Surg., Gynec., and Obst., 85:751, 1947.

Koster, H., and Kasman, L. P.: Wound disruption. Am. J. Surg., 31:537, 1936.

Lahey, M. E., Gubler, C. J., Chase, M. S., Cartwright, G. E., and Wintrobe, M. M.: Studies on copper metabolism. II. Hematologic manifestations of copper deficiency in swine. Blood 7:1053, 1952.

Larman, T. H., and Ingalls, T. H.: Vitamin C deficiency and wound healing. Ann. Surg., 105:616, 1937.

Lawrence, J. S., Pearse, H. E., and Mider, G. B.: Effect of experimental neutropenia on healing. Arch. Path., 28:32, 1939.

Leriche, R., and Policard, A.: The normal and pathological physiology of bone. Translated by Sherwood Moore and J. Albert Key, St. Louis, C. V. Mosby Co., 1928.

Levenson, S. M., Birkhill, F. R., and Waterman, D. F.: The healing of soft tissue wounds: the effect of nutrition, anemia, and age. Surg., 28:905, 1950.

Lindsay, M. K.: Observations on fracture healing in rats. J. Bone and Joint Surg., 16:162, 1934.

Lindsay, M. K., and Howes, E. L.: The breaking strength of healing fractures. J. Bone and Joint Surg., 13:491, 1931.

Localio, A. S., Casale, W., and Hinton, J. W.: Wound healing - experimental and statistical study. Surg., Gynec., and Obst., 77:481, 1943.

Localio, S. A., Casale, W., and Hinton, J. W.: Wound healing - experimental and statistical. III. Experimental observation. Surg., Gynec., and Obst., 77:244, 1943.

Localio, A., Casale, W., and Hinton, J. W.: Wound healing - experimental and statistical study. IV. Results. Surg., Gynec., and Obst., 77:376, 1943.

Logan, M. A.: Recent advances in the chemistry of calcification. Physiol. Rev., 20:522, 1940.

Lowy, P. H., Keighley, G., Borsook, H., and Graybill, A.: On the erythopoietic principle in the blood of rabbits made severely anemic and phenylhydrozine. Blood. 14:262, 1959.

Marshak, A.: Healing of experimental skin wounds. Proc. Soc. Exper. Biol. and Med., 58:63, 1947.

Marshak, A., and Byron, R. L., Jr.: A method for studying healing of bone. J. Bone and Joint Surg., 27:95, 1945.

Mason, M. L.: Wound healing. Internat. Abstr. Surg., 69:312, 1939.

McKeown, R. M., Lindsay, M. K., Harvey, S. C., and Howes, E. L.: The breaking strength of healing fractured fibulae of rats. A. M. A. Arch. Surg., 24:458, 1932.

Mitchell, H. S., and Schmidt, L.: The relation of iron from various sources to nutritional anemia. J. Biol. Chem., 70:471, 1926.

Moltke, E.: Mechanism of the action of thyrozine and scorbic acid deficiency on the T. S. of healing wounds. Acta. Endocr., KBH., 29:421, 1958.

Moore, C. V., Arrowsmith, W. R., Welch, J., and Minnick, V.: Studies in iron transportation and metabolism. J. Clin. Investigation. 18:553, 1939.

Moore, F. D., Peacock, W. C., Blakely, E., and Cope, C.: The anemia of thermal burns. Ann. Surg., 124:811, 1946.

Murray, C. R.: The timing of the fracture-healing process. J. Bone and Joint Surg., 23:598, 1941.

Newburger, B.: Early postoperative walking. I. The influence of exercise on wound healing in rats. Surg., 13:692, 1943.

Nishihara, G., and Prudden, J. F.: A quantitative relationship of wound tensile strength to length. Surg., Gynec., and Obst., 107:305, 1958.

Peyton, W. T., Anderson, U. S., and Layman, C. W.: Quantitative study of the rate of healing in bone. I. Description of method. A. M. A. Arch. Surg., 29:23, 1934.

Preston, D. J.: The effects of sutures on the strength of healing wounds. Am. J. Surg., 49:56, 1940.

Ramsay, W. N. M.: The determination of iron in blood plasma or serum. Biochem. J., London. 53:227, 1953.

Rath, C. E., and Finch, C. A.: Sternal morrow hemosiderin. J. Lab. and Clin. Med., 33:81, 1948.

Ratner, S.: Iron content of teeth of normal and anemic rats. J. Dent. Res., 15:89, 1935.

Reid, M. R.: Study of wound healing. Ann. Surg., 105:982, 1937.

Rhoads, J. E., Fliegelmann, M., and Panzer, L.: The mechanism of delayed wound healing in the presence of hypoproteinemia. J. A. M. A., 118:21, 1942.

Richardson, L. R., and Hogan, A. G.: Adequacy of milk diet for the rat. J. Nutrition. 19:13, 1940.

Robscheit-Robbins, F. S., and Whipple, G. H.: Hemoglobin production increases with severity of anemia. Am. J. Physiol., 134:263, 1941.

Sako, Y., Kremen, A., and Varco, R. L.: Measurements of healing strength of a standard wound in anemic or starvation states and after treatment with blood transfusions or refeeding. Am. College of Surgeons, Bulletin, 34:43, 1949.

Sako, Y., Kremen, A. J., Varco, R. L.: Nitrogen retention after intra-venous and oral administration of protein hydrolysate and native protein internally. Proc. Soc. Exper. Biol. Med., 68:480, 1948.

Sandblom, P.: Effect of injury on wound healing. Ann. Surg., 129:305, 1949.

Sandblom, Philip: The tensile strength of healing wounds. Acta. Chir. Scandinav., Suppl., 89; 90:1, 1948.

Sandblom, P., Petersen, P., and Muren, A.: Determination of tensile strength of healing wounds as a clinical test. Acta. Chir. Scandinav., 105:252, 1953.

Sandblom, P., and Muren, A.: Differences between the rate of healing of wounds inflicted at short time intervals. Ann. Surg., 140:449, 1954.

Savchuck, W. B.: A comparison of bone growth in normal and strontium treated rats. J. D. Res., 38:49, 1959.

Savchuk, W. B.: Effects of strontium and fluorine on the repair of unreduced humeral fractures in the adult rat. J. Bone and Joint Surg., 39 A:140, 1957.

Savlo, E. D., and Dunphy, J. E.: Healing of disrupted and resutured wounds. Surg., 36:362, 1954.

Savlo, E. D., and Dunphy, J. E.: Mechanisms of wound healing: a comparison of preliminary local and distant incisions. New England J. Med., 250:1062, 1954.

Schwartz, S. O., and Flowers, V. C.: Morphologic changes in the red blood cell with iron deficiency anemia. J. A. M. A., 130:622, 1946.

Schultze, M. O., Elvehjem, C. A., and Hart, E. B.: Studies on the copper content of the blood in nutritional anemia. J. Biol. Chem., 116:107, 1936.

Scott, E. M., and McCoy, R. H.: Iron in anemic rat tissue. Arch. Biochem., 5:349, 1944.

Shafer, W. G., Beatty, R. E., and Davis, W. B.: Effect of dilantin sodium on the tensile strength of healing wounds. Proc. Soc. Exp. Biol. and Med., 98:348, 1958.

Sheldon, W.: Hypochromic anemia with bone changes in the skull. Proc. R. Soc. M., London, 44:4 p. 298, 1951.

Shen, S. C., Ham, T. H., and Fleming, E. M.: Studies on the destruction of red blood cells. New England J. Med., 229: 701, 1943.

Sisson, R., Lang, S., Serkes, K., and Pareira, M. D.: Comparison of wound healing in various nutritional deficiency states. Surg., 44:613, 1958.

Smelo, L. S.: Problem of wound healing; effects of local agents. Arch. Surg., 33:493, 1936.

Smith, S. E., and Medlicott, M.: Blood picture of iron and copper deficiency anemia in the rat. Am. J. Physiol., 141:354, 1944.

Society for Experimental Biology: Conference on wound healing. Bull. War. Med., 4:251, 1944.

Stein, I., Stein, R., and Beller, M. L.: Living bone in health and disease. J. B. Lippincott Co., Philadelphia, 1955.

Stevens, A. R., Jr., Coleman, D. H., and Finch, C. A.: Iron metabolism: clinical evaluation of iron stores. Ann. Int. M., 38:2, 1953.

Stuck, W. G.: The effect of insulin on the healing of experimental fractures in the rabbit. J. Bone and Joint Surg., 14:109, 1932.

Symposium on copper metabolism. Johns Hopkins University, Baltimore, 1950, p. 261.

Taffel, M., and Harvey, S. C.: Effect of absolute and partial vitamin C deficiency on healing wounds. Proc. Soc. Exper. Biol. and Med., 38:518, 1938.

Teague, H. S., and Carpenter, L. E.: The demonstration of copper deficiency in growing pigs. J. Nutrit., 43:389, 1951.

Thompson, W. D., Ravdin, I. S., Rhoads, J. E., and Frank, I.: Effect of hypoproteinemia on wound disruption. Arch. Surg., 36:500, 1938.

Urist, M. R., and McLean, F. C.: Calcification of ossification. I. Calcification in the callus in healing fractures in normal rats. J. Bone and Joint Surg., 23:1, 1941.

Van Wyk, J. J., Baxter, J. H., Akeroyd, J. H., and Motulsky, A. G.: The anemia of copper deficiency in dogs compared with that produced by iron deficiency. Johns Hopkins Hosp. Bull., 93:41, 1953.

Waterman, D. F., Birkhill, F. R., and Levenson, S. M.: The healing of open wounds in the presence of anemia. Medical Nutrition Laboratory Report. 53, 1949.

Waterman, D. F., Birkhill, F. R., and Levenson, S. M.: The healing of wounds in the presence of anemia. Medical Nutrition Laboratory U. S. Army Report. 88, 1951.

Waterman, D. F., Birkhill, F. R, Pirani, C. L., and Levenson, S. M.: The healing of wounds in the presence of anemia. Surg., 31:6, p. 821, 1952.

Waddell, J., Steenbock, H., Elvehjem, C. A., and Hart, E. B.: Iron in nutrition. J. Biol. Chem., 77:769, 1928.

Waugh, W. G.: Systemic factors influencing healing. Brit. M. J., 2:236, 1941.

Waugh, W. G.: Experiments in the acceleration of wound healing. Brit. M. J., 17:249, 1940.

Weir, J. B. de. V., Bell, G. H., and Chambers, J. W.: Strength and elasticity of bone in rats on rachitogenic diet. J. Bone and Joint Surg., 31 B:444, 1949.

Whipple, A. O.: The critical latent or lag period in the healing of wounds. Ann. Surg., 112:481, 1940.

Whipple, A. O.: The essential principles in clean wound healing. Surg., Gynec., and Obst., 70:257, 1940.

Whipple, A. O.: The use of silk in the repair of clean wounds. Ann. Surg., 98:662, 1933.

Whipple, A. O., and Elliott, R. H. E., Jr.: The repair of abdominal incisions. Ann. Surg., 108:741, 1938.

Whipple, G. H., and Robscheit-Robbins, F. S.: Control basal diets in anemic dogs: method, factors and hbg. production. Am. J. Physiol., 115:651, 1936.

Williams, R. W., Mason, L. B., and Bradshaw, H. H.: Factors affecting healing. S. Forum, p. 410, 1951.

Wintrobe, M. M.: Clinical hematology. Lea and Febiger, Philadelphia, p. 1048, 1951.

Wolstenholme, G. E. W., and O'Connor, C. M.: Ciba foundation-symposium on bone structure and metabolism. Little, Brown and Co., Boston, 1956.

Young, J. S., Fisher, J. A., and Young, M.: Some observations on healing of experimental wounds in skin of rabbit. J. Path. and Bact., 52:225, 1941.

VITA

VITA

Robert Leon Ewbank

November 1, 1932	Born in Veedersburg, Indiana
1950-1954	B. S., Indiana University, Bloomington, Indiana
April 14, 1954	Married Rita Sue Maudlin
1954-1957	D. D. S., Indiana University School of Dentistry, Indianapolis, Indiana
1957-1958	Oral Surgery Intern Indiana University Medical Center Indianapolis, Indiana
1958-1959	First year Resident, Oral Surgery Indiana University Medical Center Indianapolis, Indiana
1959-1960	Chief Resident, Oral Surgery Indiana University Medical Center Indianapolis, Indiana

Professional Societies

American Dental Association
American Academy of Oral Pathology
American Dental Society of Anesthesiology
The American Society of Dentistry for Children
Indiana Society of Oral Surgeons

ABSTRACT

ABSTRACT

In the soft tissue experiment, 78 male rats of the Wistar strain were divided into 2 groups; the control group received a stock laboratory diet while the experimental group received only milk as the daily feeding. The development of anemia was measured by hemoglobin and red blood cell counts. At the end of 5 weeks, the animals were subjected to a surgical operation wherein a uniform skin incision was made in the abdominal area. Following a 5 day healing period, the surviving animals were sacrificed and the wounds evaluated on a tensile strength testing apparatus.

In the bone experiment, 74 male rats of the Wistar strain were divided into 2 groups treated as in the soft tissue experiment. The anemia was determined by hemoglobin and red blood cell counts. At the end of 5 weeks, the animals were subjected to a surgical operation wherein a uniform bur hole was placed in the tibia. Following a 7 day healing period, the surviving animals were sacrificed, the tibiae dissected free of all soft tissue and radiographed, and the wounds evaluated on a tensile strength testing apparatus.

Histological examinations made of representative animals in both soft tissue and bone studies did not reveal any significant differences between control and experimental groups. Neither could any difference be detected in the degree of healing between the control and experimental groups by radiographic means.

However, in both soft tissue and bone experiments a statistically significant difference between the tensile strength of the control and experimental wounds existed. This suggests that under the conditions of the experiment there is a relationship between the tensile strength of bone and soft tissue wounds and anemia.