Tensiometric Studies of Unwounded and Wounded Skin:

Results Using a Standardized Testing Method

WILLIAM L. WHITE, M.D., GARRY S. BRODY, M.D., ALAN A. GLASER, PH.D., ROY D. MARANGONI, PH.D., THOMAS G. BECKWITH, JAMES S. MUST, JAMES A. LEHMAN, JR., M.D.

From the Departments of Plastic Surgery and Mechanical Engineering of the University of Pittsburgh, Pittsburgh, Pennsylvania

TENSILE strength determination as an index of wound healing has interested surgeons ever since the pioneer work of Howes *et al.* in 1929.[8] Unfortunately, considerable variation in the tensile strength determinations of supposedly comparable wounds have been found by investigators using different technics.[1–4, 6, 8, 9] The vagaries of these reports suggests that there must be either a variation in the healing process or a lack of standardization in the testing technics. The wide range in results seems to indicate an error in methodology.

To solve this problem a standardized method for testing wound healing needed to be established. This report summarizes the basic technics developed and presents the results of tensiometric evaluations on unwounded and wounded skin.

Standardization of Testing Methods

Wounding Process. To minimize the variability of surgically created and sutured wounds, as shown by Geever *et al.*,[5] a machine was designed and fabricated (Fig. 1) which will produce and immediately suture a uniform lacerated wound on the backs of anesthesized guinea pigs.[12] This eliminates the variables of depth, length, angle, and provides accurate apposition of the skin edges with minimal tension.

Laboratory Animal. The albino guinea pig was chosen as the experimental animal because the animal is small, practical, and does not undergo the skin cycles common to other rodents.[11, 15] In the guinea pig, as in man, each hair follicle goes through a cycle independent of its neighbors. This is important because the physiological characteristics of skin, which includes its strength properties, is in many ways related to the hair follicle cycle.

Storage and Handling of the Test Specimen. The testing technic employed is of a destructive nature in that the tissue is removed from the living animal and tested to the point of rupture. One of the most important variables is the tissue deterioration that begins as soon as the specimen is excised from the animal. By using a quick freeze-low temperature storage technic Marangoni *et al.*[10] have shown that these alterations were minimized. This method (Fig. 2) consists of wrapping the specimen in aluminum foil, rapidly freezing the tissue with liquid nitrogen (−224° C.) and storing in a carbon dioxide chamber at −92.8° C. At the time of testing the specimens were removed from storage and quick-thawed in a water bath (24° C.).

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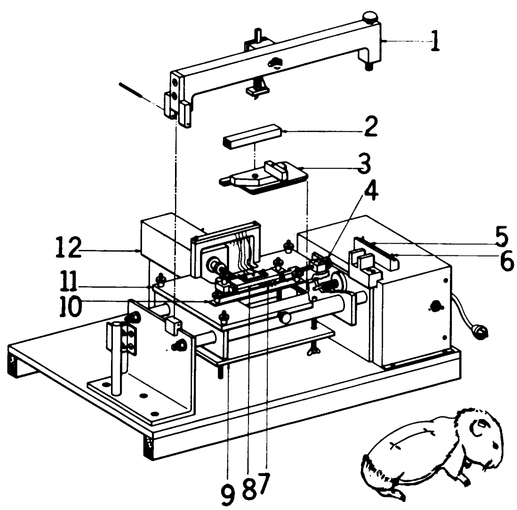

FIG. 1. Artist reproduction of machine for incising and suturing a guinea pig's back in a standard manner. 1. Cutting arm. 2. Balsa block. 3. Screw clamp. 4. Tension-turn screw. 5. Skin holder. 6. Pin. 7. Bunnell needle. 8. Needle holder. 9. Bottom plate. 10. Pressure plate. 11. Carriage. 12. Plunger.

Modified Specimen Shape. A special cutting block (Fig. 3) is used to "stamp out" the test specimens. A reduced section cutter is used to prepare unwounded skin specimens while a uniform section cutter is used for wounded specimens. In both cases, the width of the test section can be varied by making adjustments on the cutters.

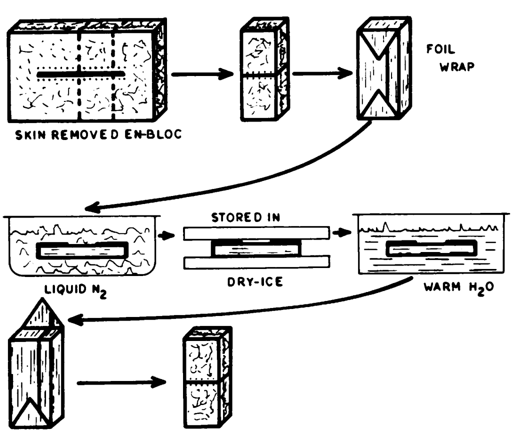

FIG. 2. The quick freeze-low temperature storage technic to minimize tissue deterioration.

Initially both the uniform and reduced section specimens exhibited a tendency to curl. The curling produces two complications: 1) photographic measurements of the test specimen used to calculate the cross-sectional area are inaccurate and 2) curling introduces a non-uniform stress distribution within the specimen.

The effect of curling was minimized by using specimens with a width equal to or less than the skin thickness.[7] Thus, the cross-section of the test specimen is nearly square, and it is observed that little detectable curling occurs under axial loading.

Methods of Experimental Measurements. The problem of making experimental measurements on skin is particularly difficult. The elastic nature of the skin makes any direct contact measurements impossible since the slightest contact pressure distorts the dimensions.[7] For this reason, photographic measurements using an overall magnification of 50× provides a practical means of determining the specimen dimensions.

After the initial photographs are taken the specimen is loaded by application of a constant strain rate. The tensile machine for doing this is illustrated in Figure 4. A continuous record of load application and specimen elongation is simultaneously recorded by means of an X-Y recorder giving a force-elongation curve (Fig. 5) from which the stress-strain characteristics can be calculated. Since skin is a complex material, it has been found that such a continuous record is necessary to record any unusual phenomena. A careful analysis of strain rates has shown that those in the interval of 0.007 in./in./sec. to 0.042 in./in. /sec. do not influence the elastic properties of skin and the results are uniform if the above recommended strain rates are used.[7]

Mechanical Parameters. From the data acquired during 6 years of tensile testing it has been found that the mechanical char-

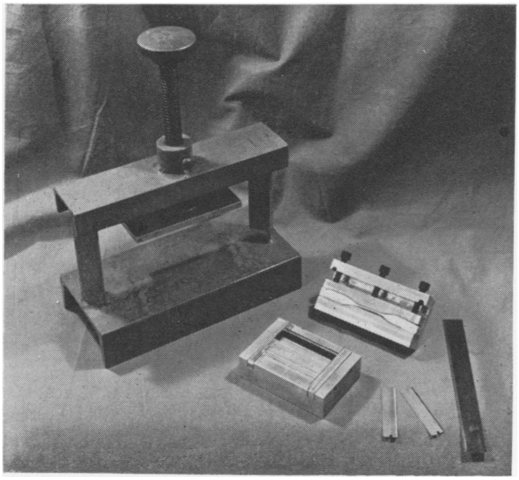

FIG. 3. Cutters used to prepare specimens for testing. Die at left is for wounded specimens while the one at right is for unwounded specimens.

acteristics of unwounded and wounded skin are more completely described in terms of four parameters (Fig. 5).[7]

Maximum Stress—This quantity is defined as the maximum "force" at rupture divided by the initial cross-section area. It gives an indication of the ultimate strength of the specimen.

Maximum Strain—The total specimen elongation prior to rupture divided by the initial specimen guage length. This gives a measure of the total "stretch" a specimen can endure without rupturing.

Maximum Work Input—This represents the work (energy) done on a specimen by the application of a force causing an elongation. This quantity is determined by calculating the area under the stress-strain curve and dividing this by the total volume of the specimen. The work input is equivalent to the total energy absorbed by the specimen.

Maximum Stiffness—This quantity gives an indication of the "elasticity" of the skin. It can be calculated by measuring the slope of the almost linear portion of the stress-strain curve. The larger the maximum stiffness, the less elastic is the skin.

Calculation of the above-mentioned parameters, therefore, permits an accurate determination of the physical properties of unwounded and wounded skin.

Application of the Standardized Method

For the determination of the normal healing process in wounds a controlled study was performed using female albino guinea pigs weighing 300–800 gm. By means of the wounding machine uniform wounds were made and sutured in the backs of anesthetized guinea pigs.

At a predetermined time the animals were sacrificed and the skin specimen stored as previously described. At the time of testing the specimens are rapidly rewarmed and cut into multiple small strips. The strips are then placed in the grips of the Instron tensile testing machine, photographs are takens, and the specimen is then distracted to the point of rupture. A continuous record of load applications and specimen elongation is simultaneously recorded by means of an X-Y recorder giving a force-elongation curve from which the stress-strain characteristics can be calculated.

Results

Typical results obtained in the studies are shown in Figure 6 through 9. These fig-

FIG. 4. Instron tensile testing apparatus with camera and x-y plotter.

ures indicate the relative strength of normal healing wounds at various time intervals as compared to normal unwounded skin.

Normal guinea pig skin has a maximum stress of 2,000 psi, while a 20-day-old wound has a maximum stress value of only 80 p.s.i. (Fig. 6). At one year this value begins to approach normal at 1,700 p.s.i. Maximum strain increases at a more rapid rate (Fig. 7) being almost one third of normal by 20 days post-wounding (0.17 in./in. vs. 0.55 in./in). Three hundred sixty-five-day-old wounds have a strain value of 0.45 in./in. which is 90% of normal. Stiffness returns to normal at a rate similar to that of maximum stress (Fig. 8). Normal unwounded skin values of 9,400 lb./sq. in. are compared to values of only 1,100 lb./sq. in. in 20 day wounds and one year values of 9,000 lb./sq. in. Figure 9 shows

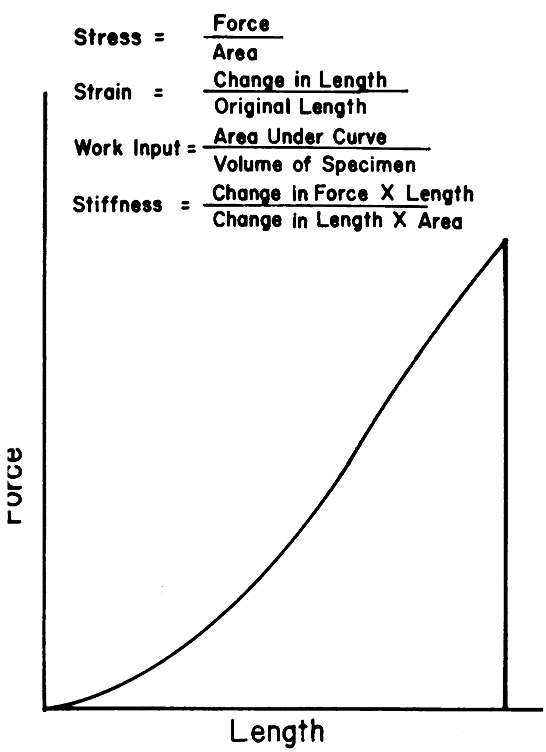

FIG. 5. Typical stress-strain curve for guinea pig skin and the physical parameters describing the mechanical properties of skin.

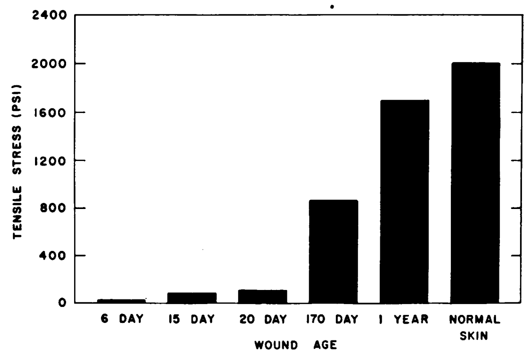

FIG. 6. The progression of wound tensile strength as a function of healing time.

that work input lags behind all the other parameters. One year values of 200 in.-lb. /cubic in. are less than 50 per cent of normal skin (430 in.-lb./cubic in.). At 20 days the work input value (10 in.-lb./cubic in.) is only a small fraction of normal.

From these results it seems apparent that previous studies indicating maximal tensile strength in wounds at 14–21 days was actually in error. In fact, 20 day wounds have but a fraction of the tensile strength of normal skin, and wounds of 365 days are then only approaching the tensile qualities of normal skin. Thus it is evident that the greatest change in physical properties occurs during a period when the mechanisms of repair are least understood.

Discussion

During the past 50 years a tremendous amount of knowledge about the basic mechanism of wound healing has been acquired, but there are still many aspects of this process which remain undetermined. Most studies in this area have been focused on the first 3 weeks of the post-injury phase during which many investigators believe the greatest activity in the histochemical and histological pattern of the wound occurs. This is a period during which only a small, relatively unmeasurable fraction of the total tensile strength gain is obtained.

This study suggests that the progression of the healing process, as determined by the aforementioned parameters, toward the pre-injury state persists for many months even though the histochemical and histological changes stabilize after approximately 21 days. This apparent prolonged maturation of the wound has only been discerned by examination of the physical properties herein described.

The value of wound healing studies based on tensile strength determinations has been the subject of considerable controversy in the literature. This has arisen in part because the methods previously employed were poorly controlled, were often extremely crude, and the results revealed great variation.

In these studies variations of two technics have been utilized. In the first, sutured skin wounds are subjected to tensiometric evaluation at predetermined times. The force causing disruption has been recorded in terms of the weight of mm. Hg[2] or water.[3,4] In the second method the abdominal wall is incised and a balloon is inserted into the peritoneal cavity through a separate stab wound. At various intervals the balloon is inflated and the pressure at the point of wound dehiscence is measured.[1] These methods utilize the measurement either of force or pressure required to rupture a wound as the indicator of wound healing. Attempts to duplicate these methods have yielded a wide variation exceeding that of biologic variation.

In view of these results it was deemed advisable to develop a refined and critical technic for testing the mechanical properties of biologic materials. Using the standardized methods previously described, the tensile properties of over 5,000 skin specimens from more than 300 animals have been recorded. These results have fallen into patterns of little variance which tends to support the value of tensiometry in the healing of guinea pig skin wounds. It also revealed a consistent long-term of maturation of wounded skin from 6 to 365 days

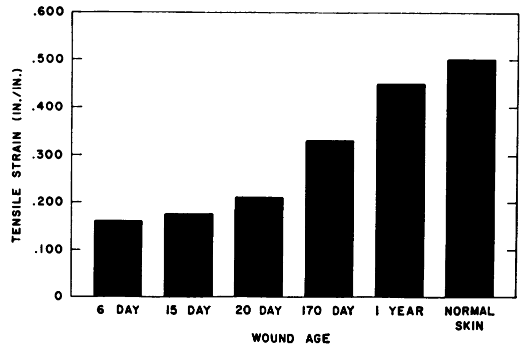

FIG. 7. Comparison of the maximum tensile strain of healing wounds at various healing periods with that of normal skin.

post-injury. Analysis of the data based on a 95 per cent level of confidence (5 per cent level of significance) have yielded results with a per cent confidence interval consistently less than 20 per cent. This attests to the reliable and reproducible results that can be obtained with these technics.

It has beeon considered an important engineering principle to measure all four physical parameters previously described. Their significance is based upon the nature of the material being tested. Because skin is a living tissue, its properties are not as uniform as most engineering materials and calculation of these mechanical characteristics gives a quantitative method for describing the mechanical properties of skin. It is possible, for example, for two specimens to have similar values for maxi-

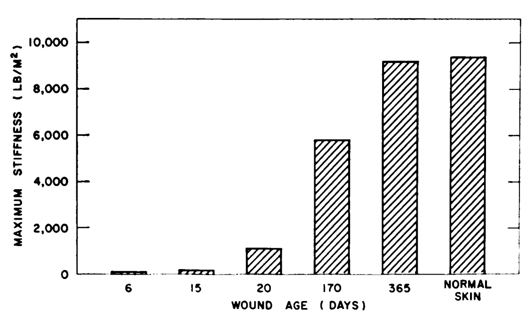

FIG. 8. The behavior of wound specimen stiffness during healing.

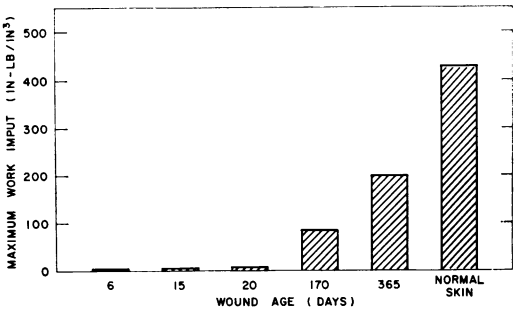

FIG. 9. Comparison of the energy absorption ability of wound specimens with regard to healing period.

mum stress and strain but different force-elongation curves. Although both specimens would have the same value for stress and strain, the remaining mechanical properties would readily reflect this dissimilarity. In addition, the calculation of these mechanical properties provides a simplified method of data analysis which is sufficiently sensitive to detect even minor variations in tissue properties.

The results in Figure 10 show that the ability of a wound to "stretch" and also its "elasticity" approach that of normal skin more rapidly than its ability to absorb energy or its ultimate tensile strength. This rapid return of elasticity allows a wound to absorb energy by stretching and is certainly an important mechanism in protecting the early wound from dehiscence. Without this rapid return of elasticity a small stress could easily rupture the wound. This may also help to explain the apparent weakness of early wounds in experimental animals when the results are based solely on the criteria of maximum stress and compared to clinical evaluations of wound healing.

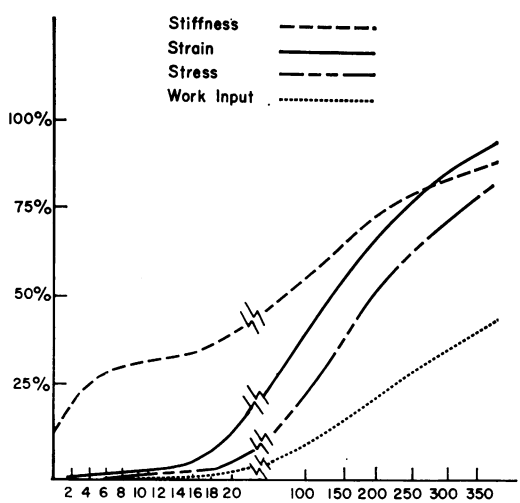

FIG. 10. Comparison of the four physical parameters showing that strain and stiffness return to normal more rapidly than stress and work input.

It appears that the maximum strain data may be of more clinical significance than the remaining parameters since all stresses (force) are introduced by means of strains (stretch) which can be accurately measured and can be used to define the functional limits of the healing wound.

The current concept of wound healing proposes that the tensile strength of a healing wound is mainly dependent on 2 factors: 1) collagen formation and 2) structural orientation at an inter and intramolecular level. Previous results have indicated that maximum collagen production is reached after 17 to 20 days.[13, 14] If this is true, then tensile properties of wounded skin are only a small fraction of those of normal skin during the time of maximum collagen production. These results tend to suggest that the combination of the inter and intramolecular cross linking mechanisms and the alteration in the architectural pattern of the collagen fibers are more important in the gain of tensile strength and mechanical properties than is the amount of collagen present in the wound. It may well be that the missing link in the further investigation of this unknown phase of wound healing will be the factor or factors involved in the different rates of maturation of these four mechanical properties. In addition, investigation of other tissues, i.e,, tendon, nerve, bowel, provides unlimited areas for research using this standardized technic.

Summary

A standardized and highly refined method for the tensiometric evaluation of skin has been presented. The results of testing over 5,000 specimens have established normal values for the mechanical properties of unwounded and wounded skin.

From the results obtained, it seems apparent that the pioneer work of Howes *et al.*[8] has been incorrectly intepreted as indicating that maximal tensile strength in wounds occurs with 14–21 days post-injury. In actual fact, the 20-day wound has but a fraction of the tensile strength of normal skin.

At present, the only mechanism for evaluating the rate and progression of wounds beyond the first few weeks after injury is through tensiometry. Continued investigation in these "uncharted waters" of wound healing may well uncover the factor or factors which influence maturation of the wound beyond the third week.

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