WOUND HEALING: BIOCHEMICAL PATHWAYS AND IN VIVO STUDIES

ANNUAL SUMMARY REPORT

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The findings of this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.
N-acetylgalactosaminyl transferase, amino acids, carbazole, carbohydrates, chondroitin sulfate, collagen, connective tissue, Dacron Weavenit vascular graft, dog, enzymes, galactosamine, glycine, glycosaminoglycans, glycosyltransferases, hyaluronic acid, hyaluronidase, hydroxylsine, hydroxyproline, ion exchange chromatography, man, oligosaccharides, proline, proteins, proteoglycans, rat, scar tissue, (continued)

The insoluble collagens from connective tissue experimentally induced by rat, were analyzed for amino acid composition. Essentially no differences were observed, the comparative values for these species being similar. In the rat it was found that these tissues contained three classes of heteropolysaccharides in a complex mixture of glycosaminoglycans, collagen disaccharides, and sialoglycoproteins as well as a less soluble fraction which is more intimately bound to the collagen fibers of tissue. The same three classes of carbohydrate macromolecules were found in the fascia adjacent to the experimentally induced connective tissue contained dermatin
No. 19 (continuation)

serum glycoproteins, sialoglycoproteins, structural glycoproteins, wire-mesh wound model and wound healing, ultrasound, acoustic impedance, compressional wave form, acoustic echoe, reflection coefficient, transducer, spectrum analyzer, buffer rod, acoustic lens, sound velocity.

No. 20 (continuation)

sulfates and chondroitin sulfates in addition. Dacron Weavenit cylinders were found to provide a unique wound model providing fresh tissue for immediate enzymatic studies.

New methodologies and biochemical procedures were established for the assay of N-acetylgalactosaminyl transferase (AGAT), the biosynthetic enzyme for chondroitin sulfate, which in turn exists in greater proportion than any of the other component glycosaminoglycans of wound tissue. The acceptor was prepared by digestion of chondroitin-4-sulfate with hyaluronidase and chromatographic isolation of oligosaccharide.

Stainless steel implanted-cylinder wound models and the healing of skin incision wounds were studied to determine variations in tissue concentrations of AGAT and hydroxyproline during the generation of wound tissue. AGAT concentrations in the wound model studies reached a maximum in 2 weeks in wound fluid and were higher than in wound tissue with a maximum at 4 weeks. In the wound incision studies AGAT concentrations reached a maximum in 1 week in wound tissue, were higher than in adjacent skin, and corresponded to the maximum concentrations of hydroxyproline from soluble collagen, also observed at 1 week in wound and adjacent tissues. The inflammatory effect of turpentine produced increased AGAT concentrations, over controls, in both wound and adjacent tissues.

Electrocautery wounds contained greater AGAT concentrations than scalpel incision control wounds. The lack of difference between the hydroxyproline concentrations of total collagen from scalpel wound and from non-wound tissue is thought to be due to the excision of too much non-wound tissue along with the wound connective tissue samples.

The velocity of ultrasound appeared to be the same in both wound and non-wound tissues samples. However, this apparent lack of expected velocity difference may be due to the small amount of sound transmitted through the narrow wound connective tissue being masked by the larger background of non-wound transmitted sound.
The insoluble collagens from connective tissue, experimentally induced by stainless steel mesh cylinders implanted in man, dog, and rat, were analyzed for amino acid composition. Essentially no differences were observed, the comparative values for these species being similar. In the rat it was found that these tissues contained three classes of heteropolysaccharides in complex mixture of glycosaminoglycans, collagen disaccharides, and sialoglycoproteins as well as a less soluble fraction which is more intimately bound to the collagen fibers of tissue. The same three classes of carbohydrate macromolecules were found in the fascia adjacent to the experimentally induced connective tissue. Only hyaluronic acid was found in the adjacent fascia, whereas the connective tissue contained dermatas sulfates and chondroitin sulfates in addition. Dacron Weavenit cylinders were found to provide a unique wound model providing fresh tissue for immediate enzymatic studies.

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There have been all too few definitive studies following the progress of the wound and at the same time correlating changes in biochemical constituents. This has been due to lack of suitable wound models. Polyvinyl sponge implants have been used extensively but leave much to be desired in the way of normal type wound tissue. The stainless steel mesh cylinder implant, in the opinion of this laboratory, more closely simulates the uncomplicated wound tissue structure. A great deal of work has been carried out in this laboratory with experimental animals and the stainless techniques and the biochemical alterations in tissue content during healing have been assayed extensively. The correlation of biochemical content with structure, function and metabolism of the wound is imperative. The use of more specific radioactive precursors of wound metabolites can and must be explored in this area. Studies of this wound model in human subjects have already been carried out successfully in this laboratory. Application of the enzymatic assay, as a postulated monitor of actual wound healing is currently under study by our laboratory.

Our earlier studies of wound healing depended almost entirely upon the adequacy of the wound model. Biochemical studies of developing connective tissue of the wound in this laboratory will continue using the stainless steel wire-mesh cylinder wound model. The model invokes the synthesis of tissue in man which appears chemically the same as that in dog or rat, and thus may ultimately aid in the diagnosis and treatment of human connective tissue diseases. Another feature of this implant model is that it provided what is considered to be a new and normal-type connective tissue which is free from the old adjacent host tissue.

We have used this simple, reproducible experimental wound model to accumulate a vast amount of information concerning wound fluid, fibroplasia, and collagen-matrix formation in rat, dog, and man. The wound is induced by a stainless steel wire mesh cylinder implanted subcutaneously into the
host with sterile techniques. The implanted cylinder itself evokes very little foreign-body inflammatory response. The size of the cylinder, mesh of the wire, and its gauge may be varied appropriately. In essence, the implanted cylinder creates a sterile dead space which fills with an extracellular plasma-like fluid and with fibrocollagenous tissue in an orderly and reproducible fashion. The wound-induced connective tissue and component fluid may be sampled from the cylinder at any time during the time-course of fibroplasia. Tracer materials such as $^{14}\text{C}$ or $^{3}\text{H}$ labeled hexosamines, hexoses, or amino acids may be injected directly into the cylinder and followed out into the whole animal body. Conversely, radioactively tagged precursors may be injected systemically and followed within the cylinder where they are incorporated or bound. The "wound fluid" inside the cylinder as well as the fibrocollagenous tissue therein may be removed and analyzed qualitatively and quantitatively for chemical content, and specific components may be isolated for radioactivity measurements. The connective tissue may also be removed fresh and intact from inside the cylinder wall and assayed by explanting into tissue culture, or it may be studied for its enzyme content or by electron microscopy and electron microscope autoradiography. The wound fluid and fibrocollagenous tissue inside the cylinder may be compared with the body fluids, blood serum, or body tissues outside the cylinder. The model is not complicated by infection, contracture, or epithelialization. It is analogous to a tissue culture in vivo of the fibroblast, a highly anabolic unit, which produces collagen and some of the glycosaminoglycans of the ground substance.

Collagen the binding tissue of all mammals is a key material in wound healing, without which there is no strength or permanence. It is not inert but shares in the dynamic balance of local and whole body stress, nutrition, and metabolism. Collagen contains a small percentage of disaccharide side-chains thought to serve both as intermolecular bridges and play
fibres are imbedded in an amorphous carbohydrate matrix. This matrix has been assigned no definite structure by present electron microscope techniques, but is known to have a complex chemical nature consisting of acidic glycosaminoglycans (mucopolysaccharides) and glycoproteins (proteins with covalently bound heteropolysaccharide chains). The matrix portion of the collagen unit is obviously important in the synthesis, maturation, and metabolism of connective tissues and the healing of wounds.

Our understanding of the intracellular location of carbohydrate attachment to the peptide portion of glycoproteins has aided studies concerning the structural makeup of these substances. The enzymes responsible for synthesis of the carbohydrate portion are located on the membranes of the endoplasmic reticulum of the cell and the assembly of the carbohydrate units take place by a series of glycosyltransferases functioning to transfer activated sugars from the nucleotide derivatives to appropriate acceptors. The synthesis of the carbohydrate units are added postribosomal but rapid physiological control can be exerted on these substances by control of enzyme activity. The absence of an enzyme of this type could result in a pathological condition, i.e., connective tissue disease and/or poor wound healing response in the host. It is obvious that the investigation of glycosyltransferases would provide an important new dimension for the studies of wound healing.

The classical methods for assessing wound healing (tensile strength, burst strength, biochemistry, histology and ultrastructure) have been extensively employed in animal studies. Each method requires some degree of wound disruption and thus has limited potential for studies in humans. Wound healing studies rely primarily on the subjective evaluation of the examiner. Methodology for objectively evaluating human wounds in a noninvasive fashion is generally unavailable. It is clear, then, that if one were able to assess the amount and nature of collagen between the wound margins (i.e. the tensile strength of the wound) in a noninvasive manner, one would have a most useful tool for assessing
wound healing in humans. To this end we have considered two modalities which are potentially adaptable to noninvasive assessment of wounds: ultrasound and controlled application of mechanical force.

The use of ultrasound is, to our knowledge, without precedent in the evaluation of scar collagen. The rationale, however, is based on a rapidly growing literature involving the use of ultrasound for characterization of other soft tissues. This study will provide the potential for comparing ultrasonic properties of scar collagen with biochemical, physiologic, and ultrastructural properties of the same tissue.

We propose to apply new technology to the study of wound healing which may permit the development of instrumentation capable of noninvasive in vivo studies in humans. An animal model will be used to provide scar tissue at various stages of maturation. Evaluation of this tissue will be performed with noninvasive techniques as well as classical standard methods for assessing wound healing. The standard invasive methods to be employed are tensile strength, testing hydroxyproline determinations, and structural morphology. The noninvasive modalities to be evaluated are ultrasound and mechanical force.

The specific aims of these studies are to contribute new knowledge, to utilize established techniques, to assay appropriate new information and to apply whenever possible this knowledge to the care and treatment of wounds. Specifically, this will involve:

1. Continuation of the biochemical studies in the rat and dog utilizing research methods developed over a number of years whereby wound connective tissue can be procured with the stainless steel wire mesh model and examined throughout its time-course of development. Also the tissue in the outer capsule of the wound model as well as tissue adjacent to the wound will be examined by the same biochemical assays and these data will be compared to that of wound tissue itself. Similar comparisons of tissue, both proximal and distal to the healing wound tissue of skin incisions will continue to

7.
be made in animals. The influence of topical agents, locally applied to the incision, can be compared with untreated incisions in the same animal. The influence and importance of the cells and tissue neighboring the wound has been neglected in this study and must now be investigated since wound connective tissue may be the end-product of these cells.

2. Examination will be made of wound tissue and surrounding tissues in the wound locale for certain enzymes which may be involved in the initial inflammatory response and in the healing phase of the wound. Measurements will continue to be made, in animals and man, of the activity of galactosaminyl transferase which has been observed to be proportional to the presence of chondroitin sulfate -- the major glycosaminoglycan component of wound connective tissue.

3. Investigation of the potential of two noninvasive modalities for assessing wound healing will be made. A dog model will be used to compare the new noninvasive modalities with invasive standard techniques. If a particular modality shows promise, an additional period of instrument refinement and testing (using a similar animal model) will ensue. When instrumentation has been developed capable of measurements which reflect the tensile strength of a healing incisional wound, the instrument will be used to study various problems in human wound healing. Another specific aim of this proposal is to provide basic information regarding the ultrasonic properties of skin and scar collagen.
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BIOCHEMICAL INVESTIGATION OF THE HEALING WOUND

A. Previous Studies (1953 - 1974)

1. Wound Model Procedure: Our wound healing studies have employed a unique, simple, and reproducible experimental wound model which develops in response to an implanted stainless steel mesh cylinder (1,2,3). The wound connective tissue, generated within multiple implanted cylinders, was discretely removed from surrounding non-wound tissues and was sufficient for multiple biochemical analyses (4). The empty space within the tissue-lined cylinder was initially filled with wound fluid, observed to be similar to serum.

2. Animal Studies: In wound fluid higher levels of albumin and glycoproteins and lower levels of high molecular weight globins were observed than in serum. Thus, the implication of glycoproteins in the wound healing process appeared possible (5).

Concomitant changes were observed in glycosaminoglycans (5) isolated, by methods developed in our laboratory (6), from collagenous wound tissue, varying in developmental age from 2 weeks to 8 months. The concentrations of the dominant glycosaminoglycan, chondroitin sulfate, and the small amount of hyaluronic acid, both were found to decrease with the age of the tissue. While the mucopolysaccharides constituted only 1/4 of the total structural carbohydrates, a part of the other 3/4 consisted of sialic acid-containing glycoproteins believed to play a dynamic role in collagen development (7).

Metabolic tracer studies were conducted in animals. When \(^{1-14}\)C-glucosamine was given intraperitoneally, it was incorporated both into acid mucopolysaccharides of wound tissue in rats (6,8) as well as into the liver (9) for the synthesis of glycoproteins which are transported to the wound site to be utilized in the synthesis of wound tissue. Injection of the labeled glucosamine directly into the wound capsule stimulated its incorporation.
into wound tissue tenfold. Glucosamine incorporation into wound connective tissue was also demonstrated in vitro and in hepatectomized rats (6). Intraperitoneally and simultaneously administered $^{35}$S-labeled sodium sulfate and $^{14}$C-labeled glucosamine, glycine, and proline demonstrated the synchronous deposition of collagen and ground substance of wound tissue in dogs (10, 11).

3. **Human Studies:** Wound tissue from cylinders implanted in human subjects was analyzed for lipid, water, dry weight, total protein, collagen, hexosamines, hexoses, hexuronic acids, and sialic acids. Analytically, there was a general similarity of the wound connective tissue from man, dog, and rat (12, 15). Depolymerization of collagen in wound tissue older than 12 weeks was noted in man but not in dogs or rats. The sialoglycoproteins of wound tissue, measured by sialic acid, remained at a constant level and were present at 3 times the amount of the acid mucopolysaccharides (glycosaminoglycans). More than half of the carbohydrate of wound tissue consisted of neutral sugars (pentoses) found in glycoproteins and the structural protein collagen and including the protein-carbohydrate linkage of several mucopolysaccharides. Part of the high content of glucose, observed in human wound connective tissue, was found in the glucose-galactose disaccharide sidechains of collagen.

REFERENCES


B. Recent Studies (1975-1977)

1. Insoluble Collagen of Wound Model Connective Tissue: Most collagen studies have been made of the soluble fraction which constitutes only a small portion of the total collagen of most tissues. Insoluble collagens from experimentally induced wound model connective tissue of man, dog, and rat were analyzed for amino acid composition (1,2). Comparative values for the three species were found to be similar. The wound tissues were observed to have higher numbers of hydroxylysine residues than the respective dermis tissues from the three species.

2. Glycopeptides of Wound Model Connective Tissue: The experimentally induced wound model connective tissue contained, in addition to collagen about 3 percent carbohydrate in the form of hexuronic acid, glucose, galactose, mannose, glucosamine, galactosamine, fucose, and sialic acid (1). The structural carbohydrate units investigated in the wound tissue of the rat (2) comprised three classes of heteropolysaccharides: glycosaminoglycans, structural sialoglycoproteins, and collagen disaccharide side-chains. A water extraction of the wound tissue removed only sialoglycopeptides estimated to be 2.3 of the total sialoglycopeptides in the connective tissue.

3. Comparison of Wound Model and Adjacent Host Connective Tissues: Biochemical comparisons were made between the connective tissues from the wound model cylinders and the tissues in the adjacent fascia. The carbohydrate portions of both connective tissues were found to consist of the three classes of heteropolysaccharides described above. The principal difference was
found in the glycosaminoglycan carbohydrate class where only hyaluronic acid appeared in the adjacent fascia while, in addition, dermaton sulfates and chondroitin sulfates were found in the wound model connective tissue.

4. Dacron "Weavenit" Wound Model: In search for an alternative prosthesis which would stimulate production of wound model connective tissue for use in a fresh state Davron Vascular graft material (3) was implanted in rats. A vascular connective tissue was produced within the Dacron tube and increased in amount with time to yield 200 mg wet tissue by 7 weeks.

5. Enzyme N-acetylgalactosaminyl transferase (AGAT) in Wound Healing:

Chondroitin sulfates are present in a greater proportion than any of the other glycosaminoglycans of wound connective tissue and thus, it was reasoned that the study of the chondroitin sulfate biosynthetic enzyme N-acetylgalactosaminyl transferase (AGAT) should serve as an index of the biosynthesis of these compounds.

Even-numbered oligosaccharides with nonreducing terminal glucuronic acid were prepared as substrate acceptors for AGAT. Whale cartilage chondroitin-4-sulfate was digested with testicular hyaluronidase and the oligosaccharide products separated by ion exchange and Sephadex gel chromatography (4).

Wound model studies were carried out in 20-35kg dogs by implanting, with a trocar, 12 stainless steel mesh cylinders in each dog into 12 separated subcutaneous pocket-areas. The cylinders, along with adjacent fascia, were removed at intervals up to 100 days following implantation. Wound fluid, encapsulated within the cavity of the cylinder, was removed immediately by syringe and needle while the inner wound tissue, encapsulating the wound fluid, was scraped from the inner surface of the cylinder.

The skin incision wound studies were carried out on 20-35kg dogs
by making 12 subcutaneous dorsal incisions, six on a side. Biopsies of the incision site, including wound tissue and adjacent dermis, were removed at intervals up to 100 days following incision.

Wet tissue aliquots were homogenized in HEPES buffer and the soluble extract assayed for the enzyme AGAT by using the radioactive nucleotide donor (UDP-N-\(^{14}\)C-galactosamine) which was transferred by AGAT to the added oligosaccharide substrate acceptor and was carried out by the method of Roden et al. (5):

\[
\text{UDP-(}^{14}\text{C)-N-Acetylgalactosamine} \rightarrow \text{oligosaccharide (acceptor)} \rightarrow \text{N-acetylgalactosaminyl transferase} = (^{14}\text{C-N-Acetylgalactosaminyloligosaccharide)}
\]

Other aliquots were extracted with acetone followed by ether, then oven dried and finally assayed for total hydroxyproline. (6)

The total yield of wound model connective tissue 4 weeks following implantation was observed to be 1.3gm (dry weight) from 12 cylinders in one dog or 1.7gm (dry weight) from 12 cylinders in six rats.

The concentrations of N-acetylgalactosaminyl transferase enzyme AGAT, measured in the wound model studies, were observed to be: highest in wound fluid, reaching a maximum by the 14th day and declining to baseline by the 42nd day; intermediate in the inner wound tissue, having reached a maximum by the 27th day; and the lowest in outer wound tissue, declining from a maximum at 14 days to baseline by 28 days.

Measurements of AGAT, in the wound incision studies, were found to be higher in the wound tissue than in the adjacent tissue, reaching a maximum by the 8th day and declining to baseline by the 22nd day.

The soluble collagen hydroxyproline concentrations, in the wound incision studies, were slightly lower in the wound tissue than the adjacent tissue and both reached maximum values at 8 days corresponding to maximum AGAT concentration in wound tissue and adjacent tissue.

The inflammatory effect of turpentine, in wound incision studies was observed to produce a concentration of AGAT in the turpentine-treated wound
twice that found in the control non-turpentine-treated wound in the same animal. A similar though smaller AGAT increase was observed for non-wound tissue adjacent to turpentine-treated wounds compared to non-wound tissue adjacent to the control non-turpentine-treated wounds.

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1. AGAT Production Following Various Kinds of Incisions: Comparisons were made of the production of the enzyme AGAT following the use of electro-cautery or scalpel to produce dermal incisions in dogs. Fourteen dorsal incisions were made in each of 4 dogs. Since laser incisions were so destructive of tissue that normal wound healing did not occur and wound...
connective tissue was not obtainable, AGAT assays were not made on these sites.

Wound healing tissues from sites of electrocautery incision were observed to have AGAT enzyme concentrations twice those found for comparable tissues at control sites of scalpel incisions, in the same dog, and 4 times those found in adjacent non-wound tissues. The AGAT concentrations in wound tissue reached a maximum in 14 days for both types of incision. The non-wound AGAT concentrations were maximal at 7 to 9 days post-incision and had returned to a baseline level at the time the wound tissue AGAT enzyme levels had reached a maximum in 14 days. The soluble collagen hydroxyproline concentrations in the wound tissues from both types of incision were found to be similar in values and reached maximum values at the same times as did the maximum AGAT enzyme concentrations for the corresponding wound tissues.

During this report period additional oligosaccharide acceptor was prepared from chondroitin sultate. To test this new acceptor batch a fresh AGAT enzyme preparation was extracted from Chick embryo epiphyses.

2. Ultrasound Assay of Incision Wound Collagen: It was considered possible to reflect both quantitative and qualitative changes in collagen during wound healing by means of ultrasound. No data are presently available on the ultrasonic properties of dermal scar collagen. However, recently the acoustic impedance of liver has been successfully related to the collagen content of the liver tissue (1). Our study was intended to compare ultrasonic properties of scar collagen with biochemical, physiologic, and ultrastructural properties of the same tissue. Scalpel incisions were made at 14 dorsal sites in each of 4 dogs. At intervals, varying from 1 to 35 days following incision, biopsies, which included the wound and adjacent dermis, were taken, trimmed of all subcutaneous fat and cut into sections appropriate for various analyses. Tissue from the incision site was immediately prepared for histology and electromicoscopy. Other wound and non-wound tissue samples for AGAT.
and hydroxyproline assays and ultrasound measurements were immediately
frozen in liquid nitrogen, and then stored at -70° until thawed for
analysis.

Total hydroxyproline concentrations, determined for dry, fat free
tissue samples, were not found to vary significantly either from 1 to
35 days following incision or between wound and non-wound tissue
(Fig. 2). The lack of these expected differences may be attributed to
the excised wound tissue sample not being narrow enough and thus including
sufficient non-wound tissue whose hydroxyproline values would mask the
small changes anticipated in the wound connective tissue. Additionally,
since total collagen, consisting of new soluble collagen and older in-
soluble collagen, was assayed, the hydroxyproline in the smaller amount of
new collagen was masked by that in the larger amount of older collagen.
Further studies are planned in which the levels of hydroxyproline
in both soluble and insoluble collagen will be followed during the
course of the developing wound connective tissue.

Sections of the skin biopsies 1 cm wide by 2 cm long and normal to
the incision were thawed and used to make ultrasound evaluations of the wound
tissue as well as the adjacent normal skin. The velocity, impedance, and
absorption characteristics of these tissues were evaluated for compressional
wave forms in an attempt to characterize the scar collagen. A substitution
technique (2) was used to measure the reflection coefficient and the long-
itudinal velocity of propagation (thus the acoustic impedance) of the skin
strip removed from the dog. In this method the spectrum of very short duration
acoustic echoes reflected from thin samples was compared to the spectrum of
echoes returned from a thick aluminum reflector of known reflectivity.
The reflection coefficient was then found from the ratios of these spectra.
It is well-known that the reflection coefficient at an interface between
two media is a function of the acoustic impedances of both media (3). If
the acoustic impedance of one medium is known, the acoustic impedance of the
other medium can be obtained from the measurement of reflection coefficient. The longitudinal velocity of propagation was calculated from the distance between two peaks of the interference pattern.

A high voltage (in the order of 100 V) spike was used to excite the transducer. The echo reflected from the sample was received by the same transducer and amplified before feeding into the linear gate. A spectrum analyzer was then used to analyze the spectrum of the gated echo. Time gating does not affect the echo spectrum if the gate width is longer than the pulse width but does allow the specified echo to be examined without spurious signals and unwanted reverberations.

To improve the accuracy we made use of the buffer rod technique (4) which is especially suitable for small samples. The sample was inserted between two buffer rods made of aluminum, which only minimally attenuated ultrasound. The transducer was attached to one end of the buffer rod. Silicon stopcock grease was used as the coupling agent between the crystal and the buffer rod. No coupling medium was used between the buffer rods and the sample. The buffer rods were pressed against the sample. The thickness of the sample was determined very accurately simply by measuring the distance between the two rods. To obtain the reference spectrum the sample was replaced by a long water path (0.9% sodium chloride solution).

The artifacts arising from the unevenness of the sample surfaces were minimized by using very narrow sound beams (3-6mm wide). This was achieved by utilizing high frequency (in the order of 15 MHz) and sharply-focused transducers.

The transducer in the buffer rod system was focused by using an acoustic lens. The material of the acoustic lens was chosen based upon the fact that its acoustic impedance should approach the geometrical mean of the acoustic impedance of the transducer and the buffer rod. In this way the reflections of ultrasound arising at the interfaces can be minimized.

Sequential ultrasound transit time measurements were made of non-wound,
wound, and then non-wound tissues as the skin strip was moved past, and positioned in, the sound beam at three different corresponding sites. These measurements were related to the velocity in saline and the velocities in the tissues as calculated by the following formula:

\[ V_T = \left[ \frac{T_S}{T_T} \right] V_S \]

\( V_T \) = velocity in tissue \((10^5 \text{ cm/sec})\)
\( T_S \) = travel time of sound in saline (micro seconds)
\( T_T \) = travel time of sound in tissue (micro seconds)
\( V_S \) = velocity of sound in saline \((10^5 \text{ cm/sec})\)

The velocities in most of the wound and non-wound tissue samples were observed to be identical and averaged \(1.5636 \times 10^5\) cm/sec, using a 6mm ultrasound beam width. However, in some tissue samples there appeared to be a marginal indication that the ultrasound velocity was greater in the wound tissue than in the non-wound tissue. The lack of expected velocity differences between the two tissues may be due to the sound beam being too large. Thus, the small amount of sound transmitted through the narrow strip of wound connective tissue may have been masked by the greater amount of sound transmitted through the larger amount of non-wound tissue simultaneously present in the sound beam. Further electronic modification of the ultrasound sensing system, in order to narrow the sound beam, is currently in progress.

**REFERENCES**

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