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THE ADHESIONS OF A TRAUMATIZED TENDON FORMED UNDER THE EFFECT OF THYROTROPIN AND SOMATOTROPIN

EXPERIMENTAL STUDIES BY PHYSICAL AND HISTOQUANTITATIVE MEASUREMENTS

by

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Henry Brummer

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1. INTRODUCTION

When one considers the development of medical science, especially that of surgery, there is no need to go very far back in time before a point is reached at which it is appropriate to state that virtually all existing knowledge had been gathered by empirical means. Figuratively speaking, each investigator describing his own cases and methods had contributed his own grain of sand to the heaped knowledge. But the higher such a sand dune of empirism becomes, the greater is the toil facing one who wants to plod through it and ascend to the top. This is because there is no solid foundation.

Traumatology is originally a by-product of military campaigns: where else, in earlier days, could one have found injuries of the most varying kind in such accumulation? Men of high morals, splendid intelligence and industry such as AMBROISE PARÉ (1517—1596) and JEAN DOMINIQUE LARREY (1766—1842) were compelled to work exclusively on an empirical basis. For this reason they were unable to give surgery any firm standing like that enjoyed by the natural sciences.

The surgeons working around the turn of this century (v. BERGMAN 1887, LEXER 1911) observed that wounds heal in one of two ways: either »per primam intentionem» or »per secundam intentionem». These concepts had originally been presented by GALEN. Even nowadays the surgeon may express his satisfaction by saying that the wound »granulates nicely», by which he means that the repair process has overcome the inflammation (BOYD 1947).

However, the actual development of surgery cannot be based on wartime surgical experience, which obviously implies reconcilement with imperfect conditions (FALTIN 1937). In our present days the increasing number of traffic and industrial accidents actually provides a more than adequate wealth of traumatological material for surgical research. This has for some time been an inducement to subject the systemic and local factors affecting the healing of wounds to experimental study, although it is true that such studies have been descriptive in character in most instances.

The formation of granulation tissue was found to be detrimental. This is clearly evident particularly in plastic surgery (GILLIES 1923), in which other requirements apply than in surgery involving the body cavities. In the extremities, the scar consequent on granulation is frequently an impediment to appropriate function. Thus, restoration surgery has appeared as a companion to traumatic surgery. Its most exacting branch is no doubt that of hand surgery, among whose promoters there may be mentioned e.g. BUNNELL (1921), ISELIN (1938) and MASON (1941).

Granulation tissue is indispensable for the repair process, but at the same time it produces detrimental cicatrization. Particularly in the complex plait work of the tendons a scar formed by connective tissue may cause adherence of the tendon to its surroundings, thus impeding its function. One of the most difficult problems in hand surgery has been to control the formation of scars and adhesions, and a great variety of expedients is employed to this end.

An altogether new possibility of controlling the scar and adhesion formation was opened up by the observation that the development of granulation tissue could be controlled with the aid of hormones. The earliest attempts to exert an influence on the formation of granulation by endocrine means consisted of glandular tissue transplantations (AIEVOLI 1923, KOSDOBA 1934), in contrast to ectomies of endocrine glands. After cortisone and corticotrophin had been isolated, these were initially successfully used against rheumatic granulations (HENCH, KENDALL, SLOCUMB and POLLEY 1949). The indications of cortisone in surgery and traumatology are about to become definitely clarified, and its application has also been tried out in hand surgery (HOWARD, BUNNELL and PRATT 1953).

In addition to cortisone, extracts of the anterior hypophysis have been found to exert a notable effect on growth and on the development of granulation tissue (EVANS and LONG 1921). Among the hormones extracted from the adenohypophysis, somatotropin and thyrotropin have considerable influence on the granulation tissue according to present concepts (ASBOE-HANSEN 1954). However, their effect on tendon adhesions has not yet been studied. The work presented in the following is thought to throw additional light also on the effect exerted by somatotropin and thyrotropin on the granulation tissue and on wound healing.

In the present work the attempt has been made to elaborate a method enabling the hormonal effects to be clarified by examination of the changes they produce in the physical properties of the tissue, together with planimetric measurements and application of histological tissue analysis, and comparison of the results obtained by different approaches.

2. REVIEW OF LITERATURE

2.1 Means employed to counteract adhesions

How to restore a tendon's ability to slide is still one of the problems which have not been completely solved. In spite of advanced surgical and operating theatre techniques the observation is sometimes made even nowadays, to the patient's and surgeon's great displeasure, that an extremity has stiffened to immobility and is unfit for use. In many instances this has been found to be due to massive adhesions, which are formed around an injured tendon and attach it firmly to the surrounding tissues, thus inhibiting its motion and frequently also that of other tendons depending on its function. In such cases the ultimate outcome of surgical reconstruction has only been worsening of the condition.

Very great pains have been taken in order that the formation of detrimental adhesions might be prevented. The expedients by which one tries to counteract their formation can be classified as follows:

(1) Methods of operating technique,
(2) Placing of an interposition,
(3) Physiatric methods,
(4) Administration of diuretics,
(5) Interference with the sympathetic nervous system, and
(6) Application of hormones.

(1) Bunnell (1921) elaborated an operating technique, which he calls the »atraumatic technique». Among other things, it implies a bloodless operating area and the use of small, smooth instruments, of suturing material that does not cause any irritation of tissues and of wetted gauze pads. Furthermore, he described the methods which have to be used in dealing with the skin, tendons and nerves. This operating technique gave rise to an entire new branch of surgery, referred to as hand surgery. It has subsequently been further developed by Littler (1947), Mason (1955), Allen (1955) and Verdan (1951).

(2) Organic as well as inorganic substances have been used as interpositions between the tendon and its sheath so that the formation of adhesions might be prevented. Henze and Mayer (1914) used sections of the peritoneum and of the vena cava for this purpose. Wilmath (1937) made experiments with the tunica vaginalis, while Weckesser, Shaw, Spears and Shea (1949) used autogenous fascia and tried out various kinds of fibrin films. The results were not satisfactory, nor is any organogenic substance being used generally.

Of the inorganic substances tested for their capacity of acting as interposition, the metal tubes surrounding the tendon may be mentioned which were employed by McKee (1945). Cellophane was tried out by Wheeldon (1939). Stinchfield (1950) used a cellulose tube and LeVeen and Barber (1949) employed various kinds of plastics such as nylon, polyethylene and Teflon. Gonzales (1949) made a comparative study of various substances used for interposition, including gelatine tubes, cellulose acetate, gelfoam and fibrin film, and with polyethylene. He found that as a rule adhesions due to foreign-body irritation and exceeding those recorded in the controls were produced in the presence of the said substances. Only with polyethylene a satisfactory result was obtained in a few cases after an immobilization period of excessive length. Moberg (1951) stresses the fact that a film enclosing the tendon will cause nutritional disturbance and necrosis, for which reason it is possible to use such films at the most on one side, e.g. as a temporary interposition against bone. Ashley, Stone, Edwards and Sloan (1960) conducted tests with cellulose filter, which was porous and permitted diffusion to take place, but this method has not been generally adopted in tendon surgery either.

(3) Physiatric methods aim in the first stage at prevention or reduction of oedema formation and, later, at obliteration of the oedema and detaching of the tendon from such adhesions as have already formed. Blair (1943) pointed out that the post-reconstructive surgery oedema is an obstacle to motion already for mechanical reasons and therefore promotes the consolidation of adhesions. He introduced an elastic compression bandage with a view to counteracting the occurrence of swelling. Moberg (1951) places weight on elevation and muscle movements as factors keeping the oedema-removing venous pump in operation. Koch (1931), Mason (1955) and Pulvertaft (1948) demonstrated both empirically and experimentally the important role played by active mobilization in restoring the tendon's sliding capacity. Counteraction of oedema and mobilizing therapy are essential aids in tendon surgery.

(4) Diuretics are used with the intention of reducing the oedematic state and the sodium retention. The best diuretics affect directly the kidneys, increasing the excretion of water and sodium and preventing the reabsorption of sodium in the kidneys. An oedematous state due to accumulation of extracellular fluid is encountered in congestive diseases of the heart, in nephrosis and in cirrhosis of the liver, all of them diseases which constitute the most important indication for the use of diuretics (GROLLMAN 1960). The effect exerted by diuretics on a local oedema produced by other causes is less pronounced, and no influence on the peripheral circulation in the extremities has been noted (MENDLOWITZ, NAFTCHI, GITLOW, WEINREB and WOLF 1960).

(5) The theory presented by LERICHE (1930) is widely known according to which pain produces a reflex in mentally sensitive individuals. By way of the sympathetic nerve tract this results is vasospasm, oedema and trophic disorders. The immobilization and articular stiffness due to pain lead to oedema, which is further acerbated by vasoconstriction. The condition is moreover aggravated by increase of the reflectory myogenic tonus. The vitious circle built up in this manner promotes the adhesion of the injured tendons to their surroundings. The school of LERICHE (1939) fights this by means of interference with the sympathetic nerve system.

(6) After the effect of cortisone, hydrocortisone and corticotrophin to prevent the formation of granulation tissue became known (RAGAN, HOWES, MEYER, PLOTZ and BLUNT 1949), clinical and experimental study were also directed on their effect upon tendon adhesions. STINCHFIELD (1950) applied cortisone locally and generally in order to counteract detrimental formation of connective tissue. GONZALES (1953) made experimental studies with dogs, flushing with hydrocortisone the tendons injured at operation. He could not observe any changes in the formation of adhesions. The extensive studies carried out by CARSTAM (1953) showed that the adhesions of rabbits' tendons treated with cortisone had lowered tensile strength. At the same time, however, he found that the tendon sutures tended to fail. ZACHARIAE and ZACHARIAE (1955) and BANG and SURY (1958) made trials with hydrocortisone treatment in Dupuytren's contracture. The opinion stated by POTENZA (1962) is thought to be prevalent that corticoids possess some significance after tenolysis operations, but that their application immediately after tendon reconstruction cannot be considered a sound principle on account of separation of the tendon fragments and the risk of infection.

Cortisone is by no means the sole hormone exerting a notable effect on granulation tissue. Despite the synergy existing between different hormones, the effect of other hormones on tendon adhesions has not been investigated.

2.2 Healing of the wound

2.21 Formation of granulation tissue

The granulation tissue coming into existence around an injured tendon conforms to the same laws as wound healing in general. This biological phenomenon has been and still is one of the central problems in surgery.

The wound area is immediately filled by coagulated blood clots and fibrin formations deposited there by the damaged blood vessels. It is not positively known what agent puts the wound repair under way. According to earlier assumptions some external stimulus would be involved (CARREL 1921). In all likelihood, however, a local stimulus seems to be concerned, arising from wound secretions or from cellular destruction (HEGEMAN, TRANT and v. WALLENSTERN 1950, ZEDERFELDT 1957). This factor set free from the cells or intercellular matter has been called »split-protein» (BIRD and MACKAY 1932). It has been said that although the primary precise stimulus is not known, the best way to evoke an ideal fibroblastic response is a clean sweep of the cold knife (DUNPHY 1963).

The process commencing in the blood and fibrin coagula accumulated in the wound is known as fibroplasia. The source has not been fully clarified from which fibroblasts are supplied to the wound. They appear there about 72 hours after the trauma (MCDONALD 1959). The fibroblasts have been suggested to come from the loose connective tissue of the organism (HOWES 1954), from the fibrous tissue cells in the adventitia of arteries and veins (MCDONALD 1959), or from the mononuclear cells of the blood (ALLGÖWER 1956). It is not possible by existing means to differentiate between the proliferative response of the capillaries to trauma and newly formed fibroblasts (GRILLO 1964). It may also be considered an established fact that new fibroblasts are not produced from differentiated connective tissue such as fascia, ligaments, dermis of the skin, or bones. Mature, differentiated connective tissue has a slow metabolic rate and is no longer able to produce young, dedifferentiated fibroblasts (Howes 1954, Edwards and Dunphy 1958).

It has been shown by tissue cultivation that fibroblasts synthetize certain carbohydrates and proteins (Dunphy and Udupa 1955). The suggestion has also been made that mast cells, which obviously synthetize at least heparin, participate in the process (Jorpes, Holmgren and Wilander 1937). A hydrophilous colloid, the »ground substance», is formed in the interstices between cells from the original exudate by synthetic action of the fibroblasts. It contains mucopolysaccharides and is held together by a network composed of proteins (Dorfman 1954, Meyer 1954).

The network holding the ground substance together consists of collagen, reticulin and elastic fibres. Of these, the reticulin obviously has the largest mucopolysaccharide component. The polypeptide molecules of the elastin are disoriented, and their chemistry is still largely unclarified (Robb-Smith 1954).

Fibroblasts have been found to be indispensable to the generation of collagen (Stearns 1940, Dunphy and Udupa 1955). It is obvious that the biosynthesis of collagen taking place in the fibroblasts includes the same biochemical processes as any other protein synthesis. The ribonucleic acids (RNA) and in the final analysis the DNA of the nucleus are thus the agents determining the course of the enzymatic reaction in the endoplasm by which collagen is ultimately produced from hydroxyproline and hydroxylysine (Dunphy 1963). This »collagen unit» synthetized by the fibroblasts is secreted into extracellular space, and only here occurs formation of fibrils (Ross, Russell and Benditt 1962). The last-mentioned authorities were able to observe collagen fibres on the surface of fibroblasts by electron microscopy (Ibid. 1963).

Investigation of the cell metabolism reveals that ribonucleic acids are formed at liveliest rate in the healing wound after five days. The rate of formation goes down after 11 days (Williamson and Duschlbauer 1963). Correspondingly, the mucopolysaccharides in the ground substance begin to decrease after five days (Kasavina, Lirzman and Muzikant 1959). After five days collagen can be demonstrated by usual histological expedients (Dunphy, Englebert and Udupa 1955). The collagen concentration increases rapidly during the period from 5 to 14 days (Jackson 1958), whereafter the process slows down.

The appearance of collagen in the granulation tissue is a sign of its incipient maturation. It is also one of the most essential events in wound healing from the clinical point of view.

2.22 *Phases of wound repair*

The healing of wounds is of utmost significance in surgical practice and in clinical research. Accordingly, this biological process is a subject of continuous, active research. CARREL and DUNOÜY (1921) observed in their clinical studies a so-called latent period in wound healing, beginning at the time of trauma and terminating when the wound starts to contract. The latent period had a duration of 5—7 days, whereafter it ended abruptly and wound contraction commenced at full force. Every surgeon has been able to learn empirically that a wound may open up again if the sutures are removed before the fifth day. FORSSELL (1960) found with human patients that a scar after laparotomy does not hold until on the sixth day; up to that time the wound is kept closed by the suture alone. WHIPPLE (1940) says with emphasis that the critical latent period, or »lag period», is the touchstone of surgery in that the wound is kept closed during this period by surgical means.

This does not imply that there would be any metabolically inactive lag phase: RAEKALLIO and LEVONEN (1963) were able to demonstrate by enzyme histochemistry the presence of adenosine + ribophosphatase activity as early as one hour after the trauma. Aminopeptidase activity was already encountered after two hours, and a couple of hours later other enzyme activities could be established as well (RAEKALLIO 1961, MONIS 1963). During the clinical lag period a biological process is gone through, of which the end result is evident in the clinical-physical ways described.

The terminology referring to a latent period or »lag phase» has been used by numerous investigators in their division of the wound healing into different phases (HOWES, SOOY and HARVEY 1925, WHIPPLE 1940, RAEKALLIO 1961, SANDBERG 1963). In experimental studies a division based on histological observations is to be preferred. Among several suggested divisions, that presented by DUNPHY and UDUPA (1955) and by EDWARDS and DUNPHY (1958) has been employed in the present work. Accordingly, the following two phases are distinguished in wound healing:

(1) the productive or substrate phase,
(2) the collagen phase.

The first phase lasts for about five days, counted from the trauma; during this time exudation occurs at first. Lively fibroblast proliferation ensues and mucopolysaccharides and soluble collagen precursor are formed.

Evidence to the same effect is furnished by abundant formation of ribonucleic acid noted after five days (Williamsson and Guschlbauer 1963).

In the second phase, after five days have passed, collagen fibres can be histologically observed in the wound. Since there is such a sharp demarcation between the productive and collagen phases, some authorities maintain that the granulation tissue present during the first phase would be completely incapable of collagen formation, for which the healthy fibroblasts in adjacent areas would be responsible (Watts et al. 1963). It is indeed a fact that in some instances, e.g. in granulomas, for some reason no collagen is formed. The collagen concentration rapidly increases during the period between 5 and 14 days (Jackson 1958). After that the growth becomes slower and gradually ceases. The wound has then attained the stage of full maturity (Whipple 1940).

2.23 Factors affecting the healing

Local factors

The factors exerting an influence on the healing of wounds may be divided into local and systemic factors. The wound is most susceptible to external influences during the productive phase of wound healing, that is, at its initial stage.

Sauerbruch (1924), Bird (1932), Bunnell (1944) and Forssell (1960) have observed that the healing of a wound is dependent on the *quantity of destroyed tissue* in the wound. It is delayed by the local, proteolytic ferments produced in destroyed tissue (Benzer, Blümel and Piza 1962). Even mere touching of the wound with a gauze pad adds to the trauma, causing slower wound healing and a greater amount of scar tissue. By means of careful measurements, Sandblom and Muren (1954) found that even such a minute circumstance of *operating technique* as the side on which the operating surgeon was sitting had a distinct influence on the healing of the wound. Bird and MacKay (1932) and Whipple (1940) stress the extraordinarily powerful effect of *local infection,* which is evident in delayed healing and increased scar tissue. Any factor affecting the *local circulation* causes slower wound healing. Sandblom and Muren (1954) noted that the method used in *shaving* the experimental animal's hair and the time at which this was done affected the wound healing. This was attributed to cooling of the skin and attendant vasoconstriction. Lundgren, Muren and ZEDERFELDT (1959) reported that vasoconstriction induced by *cold* also disturbed the healing. The experiments of BODVAL and RAIS (1962) proved that local injection of a *vasoconstrictor* distinctly impaired the wound healing. SUNDBERG and ZEDERFELDT (1959/60) noticed poorer wound healing when profuse *acute haemorrhage* had caused superficial vasoconstriction. BIRD and MACKAY (1932) observed effects exerted by various kinds of *radiation* on wound healing. FINDLAY and HOWES (1950) also found that local *oedema* increased the fibroplasia. *Mechanical or chemical irritation* of any other kind also affects the healing process (FORSSELL 1960). For instance, antiphlogistics introduced into the wound had an inhibiting effect on the granulation formation (RUDAS 1963). CARREL (1921), DUMONT (1959) and PAULETTE (1959) stated that *foreign bodies and substances* in the wound cause irritation and disturb the normal healing. This has to be taken into account especially in choosing the suturing material (BUNNELL 1944).

Systemic factors

The systemic factors affecting the healing of wounds include the age and, above all, various states of deficiency and diseases.

High *age* causes delayed wound healing (BIRD and MACKAY 1932, FORSSELL 1960), while the wounds of young individuals heal with remarkable speed. The duration of the productive phase obviously becomes longer with increasing age. After this phase has been passed, repair will progress at equal speed in young and old (HOWES and HARVEY 1932). The rate of cell division is inversely proportional to age, a fact which CARREL (1921) attributed to some inhibitory factor increasing in strength with age. TEIR and NYSTRÖM (1962) observed that skin homogenisate derived from new-born rats promoted the healing of wounds. An assumption has been presented concerning the existence of a growth-promoting factor, which becomes less with increasing age of the individual (YOUNG, FISCHER and YOUNG 1941).

The importance of *diet* with regard to undisturbed wound healing has long been known (SAUERBRUCH 1924). The retarding effect of *starvation* on the healing of wounds became evident when the experimental animals' weight had gone down 20 %. The albumin in the blood was found to decrease at this stage. FINDLAY and HOWES (1950) also stated that they observed reduced tensile strength of the wound tissues on *low-protein* *diet.* The attendant oedematic state increased the fibroplasia. DUNPHY and UDUPA (1955) noted that the productive phase of wound healing was longer in duration on low-protein diet. KOVAK, BENDITT, WISSLER and STAFFEE (1947) ascribed this to retarded collagen formation.

Disturbed *liquid and electrolyte balance* causes the productive phase in wound healing to take longer (WHIPPLE 1940). Potassium deficiency delays the healing slightly (FINDLAY and HOWES 1950). Also dehydration has been found to lower the strength of the wound (BIRD and MACKAY 1932).

Anaemia and *acute haemorrhage* have also some effect on wound healing (SANDBERG and ZEDERFELDT 1959, FORSSELL 1960).

Distinct influence of *vitamin deficiencies* on the healing of wounds has been established. SCHAUBE, CHEN, POSTLETHWEIT and DILLON (1960) observed that ascorbic acid accumulated in the wound in great quantity during the repair period; its need was strongest from the fourth to fourteenth day. ROBERTSON and SCHWARZ (1953) have found that ascorbic acid is indispensable in collagen synthesis. ROSS and BENDITT (1963) reported that extracellular collagen fibrils are not encoutered in wounds of scorbutic animals until they are given ascorbic acid.

Cytostatics have been noted to disturb but not to prohibit entirely the wound healing process (TALA and KERMINEN 1963). Changes in the rate of wound healing have been obtained with a great variety of *drugs* (FENTON and WEST 1963). BIRD and MACKAY (1932) stated that they observed delayed wound healing in certain *chronic diseases,* such as diabetes and nephritis.

2.3 The healing of an injured tendon

2.31 Histological nomenclature

A tendon is composed of collagen fibre bundles, between which the flattened cells are found. The bundles are mutually separated by the thin endotenon consisting of more delicate connective tissue. The surface of the tendon is covered by the epitenon consisting of mesothelial tissue. When the tendon runs straight, it is encircled by a loose, movable fat tissue, the paratenon, which follows the tendon in its movements. If the tendon has to make a bend, it is enclosed by the tendon sheath at such points. On the concave side of the bend originates the bifoliate mesotenon, which directly joins the epitenon of the tendon. The inner surface of the tendon sheath is lined by the peritenon, to which the mesotenon attaches. In the tendon sheath the blood vessels have access to the tendon only along the mesotenon, while a paratenoneal tendon is reached by blood vessels from all sides.

2.32 Repair of a tendon

Much speculation has been called forth by the question of how an injured tendon is repaired, because a rather highly differentiated tissue is concerned, which is a fairly specific kind of connective tissue.

(a) Seggel (1903) and Garlock (1927) considered that regeneration of the tendon proceeds from the tendon itself. As a consequence of primary, reactive hyperaemia a blood and fibrin clot is formed, which becomes organized from the tendon; the fibroblasts would then start at the tendon fragments in the endotenon and epitenon. This non-specific tissue would later be replaced by tendinous tissue coming from the tendon. The tendinous tissue itself undergoes a process in which it swells and softens at first, on the fourth day, and the contours of its cell nuclei become indistinct. At a later stage the swelling abates and the cells regain their normal appearance. – The theory ran up against difficulties when it was found that the repair of tendons enclosed in a tendon sheath progressed more poorly. Bier (1920) attributed the slow growth to a hormone-like substance contained in the synovial fluid. Wehner (1922, 1923) and Hueck (1923) were able to disprove the existence of any such specific substance in the synovial tissue. Howes (1954) and Edwards and Dunphy (1958) showed that no new fibroblasts are produced by highly differentiated tissue such as the tendon. The theory of the tendon tissue's active participation in the regeneration process has been discarded for these reasons (Skoog 1954).

(b) Mason and Shearon (1932) and Mason and Allen (1941) arrived at the conclusion that both the undifferentiated connective tissue around the tendon and the tendinous tissue itself participate in the regeneration. They divided the repair process into two phases. The first would be the connective tissue phase, at which the connective tissue within, upon and around the tendon proliferates and the fibroblasts form a collar-like scar between the fragment ends. At the second phase the tendon itself would start to proliferate after about 4—5 days. The proliferation of the connective tissue terminates after about two weeks, whereas the second phase continues and the so-called tendon callus is formed. The authors strive for analogy with the process of bone repair, by which they explain the conversion of the tendon scar into firm tissue resembling that of the tendon.

(c) The participation of the tendon in the regeneration process is altogether disputed by numerous authorities. WEHNER (1923), HUECK (1923), NÄRVI (1926), SKOOG and PERSSON (1954), DAVIDSSON (1956), ASHLEY, STONE, EDWARDS and SLOAN (1960) and POTENZA (1962) observed that at the beginning a fibrin precipitate was established between the fragments of the severed tendon. When it became organized, the connective tissue invaded it from the side. The fibroblasts grew from the paratenon outside the tendon, in between its fragments and even penetrating into the tendon, until they encountered the intact endotenon. SKOOG (1954) was able to demonstrate that only degenerative changes take place in the tendinous tissue, such as swelling and hyalinization of collagen fibrils, with associated pyknosis and karyorrhexis of tendinous cells.

Tendorrhaphy had no noteworthy effect on the repair process proper. In that case, too, the connective tissue penetrated from the side into the space between the sutured tendon fragments. At the most, suturation further lowered the vitality of the tendon.

NÄRVI (1926) noticed that the epitenon was only able to form a thin layer at the end of the injured tendon fragment, while the main regeneration took place from the peritenon and from the surrounding connective tissue.

When SKOOG (1954) damaged the surface of the tendon by removing its epitenon, strong formation of adhesions was observed and the paratenon invaded the tendon at this point too. Similarly, MASON and ALLEN (1941) admit that damage to the epitenon of the tendon produces adhesions.

The above-mentioned investigators also found that if a damaged tendon is subjected to stress at a time earlier than two weeks after the injury, increased reaction occurs at the point of trauma and heavier adhesions are formed.

WEHNER (1923) and HUECK (1923) observed that the motion of the tendon only very slowly imparts to the fibres of the scar tissue in the tendon a consistent orientation, by which the scar begins to resemble a true tendon. MASON and ALLEN's (1941) studies reveal that the tensile strength of the tendon suture in tension tests became lower within five days, after which it began to increase once more. If the tendon was not stressed at all, the tensile strength of the sutured point ceased to increase after the sixteenth day. Light stress applied from this time onwards continuously augmented the tensile strength, and by this procedure the smallest scar was also obtained. This finding is supported by the writer's own clinical observations.

2.4 Influence of hormones

In addition to the factors considered in the foregoing, also hormones have been found to exert an effect on the healing of wounds. When the influence of hormones on connective tissue are to be investigated, the fact should be borne in mind that the well-known laws governing the general action mechanism of hormones are valid in this domain too.

(1) Hormones cause no unique biochemical reactions, but they affect the intensity and rate of existing reactions. They have been likened to catalysts in this respect.

(2) In some intances a given hormone may be antagonistic to another hormone, and it may be synergistic with regard to a third one. Changes in the concentration of some hormone in the blood may therefore result in strengthened or lowered effect of this particular hormone, depending on the quantity of synergistic or antagonistic hormone that is secreted (Williams 1955).

It is obvious that in addition to the adrenal corticosteroids, thyrotropin and somatotropin have a remarkable influence on granulation tissue (Asboe-Hansen 1954).

2.41 Somatotropin

Growth hormone (GH), or somatotropin (STH), is a secretion of the eosinophilic or acidophilic cells in the so-called adenohypophysis of the anterior hypophyseal lobe. It has been known under the name of growth hormone since Smith (1927) observed that extract from the anterior hypophysis restored normal growth in hypophysectomized animals. Since growth is also greatly dependent on a number of other hormonal factors, the name has later been changed into somatotropic hormone (STH) or somatotropin. Somatotropin is one of the so-called protein hormones; its physical and chemical characteristics clearly indicate that the somatotropin preparations obtained from the hypophyses of different animal species are different proteins. The somatotropins of the primates have the lowest molecular weight and isoelectric point, but not even the protein hormones of as closely related species as man and ape are identical (Li 1960).

Metabolic effects. The metabolic effects of somatotropin concentrate on the protein metabolism, calcium metabolism, sodium and chloride metabolism, fat metabolism, ketogenesis and carbohydrate metabolism as their targets. The effect on the protein metabolism is anabolic, causing retention of nitrogen, phosphorus and potassium. Somatotropin affects the calcium metabolism by increasing the calcium excretion with the urine and accelerating the metabolism of bone (DENKO and BERGENSTAL 1955). Retention of sodium and chlorides occurs during somatotropin administration, and the intercellular fluid quantity increases. Fat is mobilized in the tissues under its effect. In the carbohydrate metabolism a diabetogenic factor is encountered in somatotropin, which aggravates the disease of diabetics (IKKOS, LUFT and GEMZELL 1958).

Biological effects. Somatotropin produces remarkable increase of growth in hypopituitary dwarfs (BLIZZARD 1963). The blood plasma of children and subadults has been found to contain twenty times as much growth hormone as that of adults (GREENWOOD, HUNTER and MARRIAN 1964). Growth hormone is reported to stimulate the formation of granulation tissue in infected tissues (TAUBENHAUS and AMROMIN 1950). In mycotic infections of rats, CAVALLERO (1953) observed stimulation of connective tissue formation by somatotropin, resulting in powerful connective tissue proliferation around the infected tissue. No effect on the agent responsible for the infection, such as the tuberculosis bacillus, was observed by WASZ-HÖCKERT (1963). According to WILLIAMSSON and NEUMAN (1954), small somatotropin doses appear to be optimal, whereas larger doses cause inhibition. SPAIN and MOLOMUT (1953) did not succeed in producing stimulation of granulation tissue in mice with somatotropin. Somatotropin did not increase the growth in tissues inhibited with cortisone. KOKKONEN (1963) actually noted initial decrease of the connective tissue formation in a tuberculous gland. On the other hand, TAUBENHAUS, TAYLOR and MORTON (1952) established a stimulating effect of somatotropin in granulations which had been subjected to inhibition by cortisone.

TAUBENHAUS (1950) found that in rats to which somatotropin was given, granulation tissue was formed more abundantly in turpentine abscesses than in normal rats. The walls of the abscesses became thicker, the fibroblasts were large and multipolar. Multinuclear giant cells also occurred. Pale, homogeneous collagen was abundant.

Specific effects on granulation tissue. WEGELIUS and ASBOE-HANSEN (1956) observed, under somatotropin effect, increased formation of mucopolysaccharides as a result of mast cell stimulation. TAUBENHAUS and AMROMIN (1950) found that somatotropin stimulated the fibroblast proliferation. SCOW (1951) observed in thyroidectomized rats increase in collagen formation in skin and muscles. It should be noted that thyroidectomy considerably accelerates the thyrotropic hormone secretion of the hypophysis and that many somatotropin extracts are thyrotropin-contaminated (ASBOE-HANSEN 1959). BANFIELD (1958) studied with hamsters the collagen extracts of skin and found that the acid-soluble collagen quantity increases with age. Daily somatotropin injections increased the collagen concentration. BANFIELD discussed the question whether the increase was due to change of old or precipitation of new collagen and whether the effect of somatotropin was a direct one or was produced by mediation of one or several endocrine glands. SAIKKU (1956) observed with rats that somatotropin at first increased the quantity of granulation tissue but that the difference with regard to the controls levelled out in more mature granulations. To start with, the granulation tissue was similar to that of the controls, but at a later time collagen fibrils occurred in greater and intercellular substance in smaller amount than in the controls. GYLLING (1964) elicited no changes with somatotropin injections administered to rats in the intraabdominal adhesions which had been induced in them. Only in combination with thyrotropin was ground substance produced in considerable quantity.

Effect on bone. LENTIN and SUMMA (1958) observed that somatotropin injections promote the formation of new bone in fractures. This was attributed to its action on cartilage. Somatotropin appears to have chondrotrophic effect. KOSKINEN (1959) found that somatotropin caused powerful osteogenesis in the reparative phase of experimental fracture repair, resulting in rapid callus formation and maturation of the callus tissue. In studies by electron microscopy of the cartilage developing under somatotropin effect, SILBERBERG et. al. (1964) observed hyperplasia and hypertrophy of chondrocytes and accelerated ossification. They call particular attention to the fact that the fibrillar content of the matrix increased and the development of the collagen fibres was accelerated. The cartilage matured and aged, as evidenced by disorientation, »asbestosis», of collagen fibres. In their discussion of the problem, they ascribe the phenomenon to strong metabolic activity of the fibrocytes, i.e., to an increase in quantity of the cellular products. At the strengthened protein synthesis hydroxyproline would become incorporated and the formation of collagen precursor would increase in intracellular space.

Immunology. The chemical differences between the somatotropic hormones from different animal species are also evident in their biological effects when hormone of another species is administered. Immunological studies reveal that protein hormones produce species-specific antisubstances. Owing to their effect, the somatotropin preparations available at present have no effect on the rabbit, and in the guinea-pig they produce anaphylactic shock. Man has also been found to become immune to somatotropin from steers (Komer, Randle and Young 1959, Hunter and Greenwood 1962). The rat, again, is able to react to somatotropin preparations derived from a great variety of animals (Li 1960). It has not yet been clarified whether this is because the protein hormones of different species have a common, biologically active nucleus (Wilhelmi 1955) or whether the rat has an enzyme system which enables it to evaluate somatotropin obtained from various species (Li 1959).

2.42 *Thyrotropin*

Thyrotropin or thyrotropic hormone (TTH) is probably a secretion of the basophilic cells in the anterior pituitary lobe, the so-called adenohypophysis. A water-soluble substance, it is difficult to isolate in the pure state (Morris and Fawcett 1960). According to Williams (1955), thyrotropin is a protein hormone having a molecular weight slightly less than 10,000 and an isoelectric point in the range of 8.0—8.5.

As the name implies, thyrotropin exerts a thyroid-stimulating effect, but its direct influence on connective tissue has also been observed. It becomes manifest in the generation of exophthalmus. In currently available preparations two components can be distinguished: the exophthalmus-producing hormone (EPH) or ophthalmotropic hormone (OH) and the thyroid-stimulating hormone (TSH) or thyrotropic hormone (Brunish 1960). They cannot yet be obtained free from each other. The thyroxin hormone secreted by the thyroid gland has an inhibitory effect on hypophyseal thyrotropin secretion. When thyrotropin is administered to intact experimental animals, this regulatory mechanism causes inhibition of the thyrotropin effect.

Metabolic effects. Iversen and Asboe-Hansen (1952) found that thyrotropic hormone caused in guinea-pigs hyperlipaemia and rapid transportation of fat from its normal places of storage to muscles, liver and kidneys. A direct effect of thyrotropin is obviously concerned, because thyroidectomy has no influence on the phenomenon (Jeffries 1949).

In human studies, SKANSE and VON STUDNITZ (1962) elicited no metabolic effect of thyrotropin in thyroidectomized patients. The increase of basal metabolic rate and of erythrocyte sedimentation rate noted in their tests was attributed to the thyroid-stimulating influence of thyrotropin. The effect of thyrotropin on the normal organism was similar to the state encountered in infections. This could also be observed by LAMBERG and GRÄSBECK (1955) in hyperthyroidism.

Biological effects. ASBOE-HANSEN (1959) observed that on administration of thyrotropin the fat in the tissues is replaced by a mucinous substance, which belongs to the acid mucopolysaccharides and is mainly hyaluronic acid. LUDWIG, BOAS and SOFFER (1959) found that mucopolysaccharides accumulate and hyaluronic acid increases in the retrobulbar tissue under the effect of thyrotropic hormone. The accumulation of water-retaining hyaluronic acid in the said tissue probably increases the volume of the orbital tissue and causes exophthalmus (DOBYNS and STEELMAN 1953). The effect of thyrotropin is opposite to that of the glucocorticoid hormones in many respects.

WEGELIUS and JOKINEN (1962) achieved with thyrotropin not only an increase in the quantity of mucopolysaccharides but also increased amount of connective tissue cells. Stimulation of thyroid tissue but not of old, mature connective tissue by thyrotropin *in vitro* was reported by BERGMARK, HEIMAN and TENGROTEN (1964). Thyrotropin has also been found to exert an influence on the growth of cartilaginous tissue (THOMPSON and CREAN 1963). GYLLING (1964) produced with thyrotropin an increase in the relative ground substance quantity in connective tissue. This phenomenon indicating immaturity of the connective tissue was highly distinct when thyrotropin was given together with somatotropin.

Immunology. FRIEGOOD (1934) observed that the metabolism-accelerating effect of hypophyseal extract ceased in rats after four weeks and later administration of anterior pituitary extracts had no effect. WERNER, SEEGAL and OSSERMAN (1961) were in fact able to produce antiserum to thyrotropin from steers by hyperimmunizing rabbits. A specific, precipitating antibody was then formed. The antisera were able to neutralize the thyrotropin effect. In man, SKANSE and STUDNITZ (1962) have found the metabolic effect of thyrotropin extracts to vanish after two weeks' hormone medication. Hormone given after this period did no longer affect the metabolism.

3. PROBLEMS

The facts reviewed in the preceding chapters reveal that the regeneration of tendons and the formation of adhesions in the course of the process are still rather incompletely known in many respects and that little actual knowledge exists concerning hormonal effects in this connection. It seemed appropriate to undertake a study aiming at clarification of the manner in which adhesions are formed between a traumatized tendon and its surroundings under the effect of thyrotropin and somatotropin.

In planning this study, endeavours were made to enlist such methods of investigation the results of which can be expressed by numerical values and examined for mutual correlation. This enables comparisons to be made between the results found by the different methods and the findings to be checked both empirically and mathematically. On such reasoning the following quantities were chosen to constitute the objects of the present study: tensile strength and elongation of the tendon adhesions, cross section of the adhesions, and data obtained by histological tissue analysis.

On the basis of the foregoing, the following questions were considered to require particular attention:

(1) What effect is exerted by thyrotropin and somatotropin on the tensile strength and elongation of the adhesions formed around a traumatized tendon?

(2) What is the effect of the said hormones on the cross section area of these adhesions?

(3) What changes are caused by the said hormones in the histological picture presented by the granulation tissue developing around the tendon?

(4) What correlations, if any, are demonstrable between the results yielded by the three methods of investigation mentioned?

(5) Are the results obtained in answer to the questions examined here also applicable in general when other instances of formation of granulation tissue under hormonal effect are concerned?

4. MATERIAL AND ARRANGEMENT OF THE WORK

4.1 Experimental animals, their selection and handling

For reasons of operation technique it is convenient to choose a large-sized animal species to be used in experimental tendon studies. HUECK (1923), MASON and ALLEN (1941) and GONZALES (1953) used for this purpose the dog, which has large, well-developed and easily accessible tendons, covered as they are by a thin subcutis. In extensive series tests, however, it is difficult to assemble a material of uniform size and age of this animal species. Studies of this kind have therefore been made, for instance, with the cat (NÄRVI 1926) and rabbit (CARSTAM 1953, SKOOG 1954), whose extensor hallucis longus tendon seems to be well appropriate for the purpose in question.

However, since the present work involves the effect of protein hormones such as somatotropin and thyrotropin, the rat was chosen as experimental animal in the study to be reported in the following. The choice seems advisable in the light of previously presented immunological factors (LI 1960). Moreover, adequate control references are available concerning experimental work carried out with the rat under the effect of the said hormones; it does not concern the tendons but still the mesenchymal tissue (TRAUBENHAUS 1950, CAVALLERO 1953, SCOW 1951, SAIKKU 1956, KOSKINEN 1959).

In the handling of the experimental animals all known systemic factors of influence on the healing of wounds were taken into account as carefully as possible. The experimental animals were young albino rats of equal age, four months. All belonged to the same Wistar strain. Male rats were exclusively used, their body weight varying between 210 and 320 g. Only completely healthy animals in good condition were included in the tests. They were kept in a draught-free, airy and dry room in natural light. Electric light was only occasionally used. The temperature in the room was maintained at a uniform 20°C. Ten rats were accommodated in each cage and they were returned to the same, familiar cage after the operation. Care was taken of the animals' protein, mineral, vitamin and fluid requirements by feeding them a commercially manufactured special diet (Hankkija's feed mixture for mice) and giving them fresh milk and water ad lib.

4.2 Anatomical considerations

The tendon constituting the object in experimental tendon studies should be easily accessible and isolable from other tissues by operation. In order to facilitate the operation, the tendon should be located close to the surface, free from muscles, and it should have uniform structure (GONZALES 1953). Even though the rat is a small animal, its Achilles tendon was found to comply with these requirements. With its length of only 2 cm it is comparatively short for complicated tendon operations, but the length was found to be sufficient for the intended adhesion studies.

The Achilles tendon, located in the distal part of the lower leg on its posterior side, lies in the rat under a nearly hairless or very short-haired skin with scanty subcutis. Medial to it lie the tibial nerve and posterior tibial artery, which are easily separable from the tendon. Immediately under the tendon, the tibia and the ligamentous structures of both ankle joints are encountered. The tendon itself consists of two bundles, the gastrocnemius and soleus muscles, which both attach to the calcaneus. Although according to BUNNELL (1944) tendons possessing a peritenon chiefly occur only in places where the tendon has to make a bend, microscopic examinations revealed that the tendon has a peritenon and mesotenon close to the calcaneus. It seems obvious that nutritional circumstances are decisive in this respect, seeing that the movable skin covering the tendon carries few blood vessels at this particular point.

4.3 Operating technique

The indispensability of most painstaking accuracy in performing the operation and in traumatizing the tendon has to be stressed. The local factors affecting the healing of the wound have to be carefully taken into account in order to ensure highest possible uniformity of the trauma.

Keeping these aspects in mind, traumatization of the Achilles tendon was effected in the following manner. The animals, which had been denied any food during eight hours prior to the operation, were anaesthetized with ether. Both lower extremities were scrubbed with soap and water. The skin was swabbed with alcohol, and the animal was then placed on its stomach on a sterile support. The operating area was covered with an operating cloth wetted with sodium chloride solution, in which small holes were made at the points of entry. Since the skin was nearly hairless or carried very short hair where it was to be pierced, it was not considered worth while to traumatize it by shaving the hairs.

The operation was performed under strictly aseptic conditions, using instruments sterilized in the autoclave. An assistant held the anterior part of the animal's body with one hand and the extremity to be operated upon with the other, bending it to a right angle at the ankle joint over the edge of the operating table. Since no operating lamp with filter was available, merely indirect, reflected light was employed. The operation on the right lower extremity was invariably performed first. Beside the Achilles tendon, on its lateral side, an incision 1.5 cm in length was made parallel to the tendon. Due to the practice of following an atraumatic operating technique, virtually no bleeding occurred. The subcutaneous fascia and the peritenon were opened at an acute angle, excercising care not to touch the tendon. The peritenon was drawn aside at an obtuse angle to the medial side, by which the tendon was exposed. The tendon was lifted into view with a round, smooth instrument.

Traumatization was performed at a point 1 cm from the attachment of the tendon to the calcaneus. The tendon was placed between the jaws of a clamp 5 mm in width and the clamp was tightened with the same amount of force in every instance. This produced a flattening of the tendon so that distinct impressions could be seen in the epitenon on macroscopic examination (Fig. 1). The tendon was then allowed to settle back in its proper position. No attempt was made to close the peritenon. The skin was sutured with five-zero atraumatic silk stitches spaced at 3 mm, and the wound was covered with a Nobecutan film.

Exactly the same measures were then performed on the left Achilles tendon. No expedients for draining blood from the wound or for haemostasis were necessary. The entire surgical interference took only five minutes per extremity. The animals were left to recover from the anaesthesia in individual boxes and were ultimately returned to the previous cage housing their particular group. Each operation series, together with its controls, was put through the operation on one and the same day.

It should ne noted that the traumatizing of the tendon included no cutting of its fibres, nor was any kind of tendorrhaphy undertaken. This was in the interest of highest possible uniformity of the trauma produced in each tendon. It is not fully consistent with the trauma most commonly ocurring in operative tendon surgery. In previous experimental tendon surgery the trauma has been made to consist of severing and resuturation of the tendon in cases in which prevention of the adhesion by means of interposition has been attempted (Henze and Mayer 1914, Wilmath 1937, Weckesser, Shaw, Spears and Shea 1949, McKee 1945, Wheeldon 1939, Stinchfield 1950, LeVeen and Barberia 1949, Gonzales 1949 and Ashley, Stone, Edwards and Sloan 1960).

The tendon was severed also by Wehner (1923), Hueck (1923), Närvi (1926), Mason and Allen (1941), Ashley, Stone, Edwards and Sloan (1960) and Potenza (1962) in their studies on the recovery mechanism of the tendon. In his work concerning the adhesions that are formed around a tendon, Carstam (1953) applied a traumatizing procedure in which the surface of the tendon was rubbed with a piece of dry gauze, whereupon the tendon was squeezed at two points with artery clamps and finally one-quater of the tendon's fibres were severed by incision at three points. Mason and Allen (1941) concede, however, that already damage to the epitenon of the tendon causes adhesions. In SKOOG's (1954) studies injury of the epitenon was found to cause strong formation of adhesions and fibroblast invasion from the surrounding tissues into the tendon.

Preliminary studies performed by the writer showed that mere contusion of the tendon and damage to its epitenon were sufficient to provoke the formation of adhesions. More complex traumatizing procedures are accompanied by a great number of local factors influencing the healing of the wound, such as tissue destruction, risk of infection, foreign body irritation, haemorrhage, cooling of tissues, radiation, etc., each of which contributes in its own manner to the course of the healing process. For this reason the traumatization described above is thought to be preferable, while it is adequate and reliable at the same time.

4.4 Experimental groups

Subsequent to operation the experimental animals were immediately divided into four groups.

Fig. 1. Traumatized tendon, photographed during operation. The impressions left in the epitenon by the clamp can be seen.

(I) *Control Group* (subsequently referred to by the symbol C). The animals in this group were given an i.m. injection of 0.1 cc. physiological saline into the muscles of the back immediately after the operation and thereafter once every day.

(II) *Somatotropin Group* (STH). In this group an i.m. injection of 25 tibia units somatotropin (»Somacton» of Ferring Ab., Malmö, Sweden) into the muscles of the back was administered immediately upon operation and subsequently once every day.

(III) *Thyrotropin Group* (TTH). In this group an i.m. injection of 0.1 U.S.P. units thyrotropin (»Actyron» of Ferring Ab., Malmö, Sweden) into the muscles of the back was administered immediately upon operation and later once every day.

(IV) *Somatotropin Plus Thyrotropin Group* (STH + TTH). In this group the animals received simultaneous i.m. injections of 25 tibia units somatotropin and 0.1 U.S.P. units thyrotropin into the muscles of the back immediately upon operation and subsequently once every day.

Each group was subdivided into two subgroups. The animals of the first subgroup were sacrificed after five days and those of the other after 14 days. The sacrificing procedure was simple decapitation.

The above-mentioned sacrificing times had been fixed on the basis of considerations concerning the healing of the wound. It was to be expected that the productive phase of the wound repair process would terminate at five days after the operation, when the collagenous phase would begin (Dunphy and Udupa 1955, Edwards and Dunphy 1958). At 14 days the rate of the process in the collagenous phase of repair slows down considerably (Jackson 1958). Preliminary tests had also shown that five days and 14 days were the most appropriate times of examination.

4.5 Statistical analysis

Presentation in detail of the methods of statistical mathematics employed in analysis of the results obtained in the present study is not thought to be indicated, and reference to pertinent text-books (e.g. Cramér 1946) may suffice. It shall merely be mentioned that in applying Student's *t* test a highly significant, significant or almost significant difference has been stated to exist when the probability for the deviation between two sets of values to be due to chance and not actually true was less than 0.001 (1 %), less than 0.01 (1 %) or less than 0.05 (5 %), respectively, on the strength of the test.

5. TENSION TESTS

5.1 Procedures of tensile strength investigation

5.11 Characteristics of tensile strength and their investigation in physics and technology

The nature and structure of physical substances and also of organic tissues determine their physical properties. Changes in structure, among others, are reflected as changes of the strength characteristics. These are values describing the behaviour of the material when it is subjected to stresses produced e.g. by the action of external forces. The principal modes of stress are tension, compression, shear, bending and torsion stress, concerning each of which specific theories have been elaborated. Within each category a difference has to be made between static and dynamic stress. The latter means that the direction of the force continuously alternates, and failure under dynamic stress has mostly the character of a fatigue phenomenon.

The forces to which a tendon attached to its enviroment by adhesions is subjected vary incessantly in direction. A condition of dynamic stress would thus seem to be concerned. From MASON and ALLEN's (1941) studies the inference can be drawn that the dynamic aspect is rather significant in practical surgery from the viewpoint of the development of adhesion tissue. However, experiments involving static load, i.e., with constant direction of the acting force, are much less elaborate than dynamic strength examinations and relevant conclusions concerning the behaviour of the subject can also be drawn from them.

If a body or a piece of tissue is subjected to the action of an external force, if it is placed under load, the force tends to change the mutual positions of its particles or to detach them entirely from each other. The amount of deformation is usually proportional to the magnitude of the force up to a given limit, the elastic limit. When this limit has been passed, the deformation is no longer reversible. In medical applications this can be considered equivalent to the point at which traumatic effects are elicited, the trauma limit.

The behaviour of a great number of materials under tension or compression is characterized by *Hooke's law*. According to this law, the elongation of a bar or wire under tensile load or its compression under compressive load, λ, is proportional to the initial length, l, to the magnitude of the deforming force, F, and to the inverse of the cross section A of the bar or wire. This is expressed by the equation

$$\lambda = \frac{F \cdot l}{E \cdot A}$$

where E is a characteristic constant of the material and is called its modulus of elasticity. The quotient $F/A = \sigma$ is the stress acting at every cross section of the object under load. When the load is increased to a certain limit, the yield limit, Hooke's law ceases to be valid: the deformation increases without further increase of the load. Later, still, the breaking limit is reached, at which the specimen fails by rupture. The value of the stress at which this occurs, σ_u, is called the ultimate, tensile or compressive, strength of the material.

In virtually all branches of technology strength tests are applied as routine in order to control the products' compliance with specifications, and in research. Changes of physical and chemical properties are rather sensitively reflected e.g. by the tensile strength value, and for this reason tensile strength determinations are practised e.g. in the textile and paper industries as a means of controlling the uniformity of the output. When the durability of yarns is tested, it is usual to determine the ultimate elongation in addition to the ultimate tensile strength, by which a more complete picture is obtained of the yarn's properties.

The tensile strength testing procedure suggests itself as a suitable expedient also when it is desired to observe the physiological and pathological changes occurring in organic tissues. In such previous studies in which this has been done, the methods and equipment have mostly been borrowed from the textile or paper industry, which have to deal with strength characteristics and dimensions roughly in the same order of magnitude. It seems appropriate to present a brief review of the equipment of the said kind employed in the above-mentioned branches of technology.

(1) A balance has been used, adding weights or a length of chain into one of its pans (Bowman, Matthews, rev. by Skinkle 1949) or pouring water into it (Krais, rev. ibid.). The pan may also be pulled down by an electromagnet (Barratt, rev. ibid.), or the load increase may be achieved by allowing water to flow out of a cylinder (O'Neil, rev. ibid.). Devices of this kind are obsolete and they have been discarded owing to their inaccuracy and iconvenience and because they are slow in operation.

(2) In pendulum-type testing machines a pendulum is deflected from its equilibrium position by means of a force applied through the yarn to be tested. The ultimate strength is determined from the position of the pendulum at the moment when the yarn breaks. The load is applied by turning a hand wheel, by means of an electric motor or with the aid of a hydraulically controlled weight (H. BAER, rev. ibid.). Testing machines of this kind are rapid, practical and robust. They are manufactured in several sizes for various applications.

(3) Independent of the manner in which the loading force is generated and controlled, its magnitude can be ascertained on the basis of the change in electrical resistance a thin metal wire experiences under the load, this change being proportional to the acting force (the so-called strain gauge principle).

(4) In another class of devices a constant weight is placed on a carriage on rails. When the rails are slowly tilted, the force tending to put the carriage in motion gradually increases. The accuracy of devices of this kind is good owing to the fact that the length, elongation and cross section area of the specimen have no influence on the magnitude of the acting force. A great variety of tests can be carried out with one and the same machine, in addition to which its capacity is variable within wide limits by using different weights. However, the testing procedure is comparatively slow. The device has not come into general use in industry for this reason.

(5) Ballistic devices, in which a pendulum is made to break the yarn that is tested and the change in its amplitude of oscillation is observed.

5.12 Previous tensile strength studies in medical research

In their studies concerning the factors affecting the healing of wounds, the following authorities have been among those who employed tension testing machines of the kinds described above: SANDBLOM (1944), FINDLAY and HOWES (1950, 1952), JACKSON (1954), SANDBLOM and MUREN (1954), DUNDPHY, ENGLEBERT and UDUPA (1955), SAIKKU (1956), ZEDERFELDT (1957), LUNDGREN, MUREN and ZEDERFELDT (1959), SANDBERG and ZEDERFELDT (1959/60), and KULLANDER (1962). Tension tests with tendinous tissue in particular have been carried out by NICOLA (1934), CRONKITE (1935/36), GRAZ (1937), MASON and ALLEN (1941), CARSTAM (1953), GONZALES (1953), MOBERG and STENER (1953) and DAVIDSON (1956).

The writer's preliminary studies revealed, however, that tension testing machines of the types common in industrial use are not well-suited for tissue studies. Such machines are required to be rapid, sturdy and practical, and they have been designed for application in closely circumscribed tasks. Pendulum devices, in particular, are impractical owing to their small capacity. Even when used in their originally intended application, they introduce an error of about 2.5 % owing to friction and inertia, according

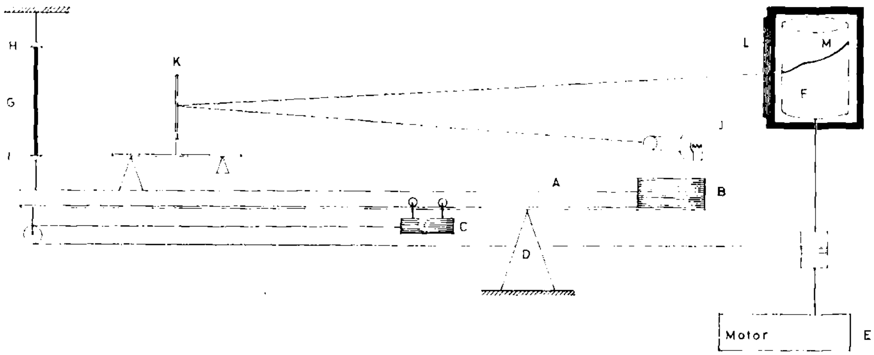


Fig. 2. Principle diagram of the tension testing apparatus. See text for detailed description.

to SKINKLE (1949). Further errors are caused by the design of the clamping jaws, the length and the elongation of the test specimen. The results are also affected by the rate at which the lower jaw moves and its uniformity. Hand-operated machines in particular are unreliable in this respect, yielding rather arbitrary readings. For the above-mentioned reasons a special tensometer has been elaborated for use in the study of skin wounds (SANDBLOM, PETERSEN and MUREN 1953). The device is also suitable for clinical use, but it is not quite as appropriate for the determination of the tensile strength of tendinous adhesions.

5.13 Design and operation of the tension testing machine

The following characteristics are desirable in a tension testing machine to be applied in studying the tensile strength of organic tissues and particulary of tendon adhesions:

- Minimum friction and inertia;
- Clamping jaws allowing no slipping, yet producing no damage of the tissue;
- Results to be unaffected by the length of the specimen;
- Constant sensitivity with changing load;
- Possibility of changing the capacity of the device without change of sensitivity;
- Automatic recording;
- Determination of ultimate tensile strength as well as elongation shall be possible;
- Elongation and load shall be determinable as each other's functions (i.e., the stress-strain curve shall be obtainable).

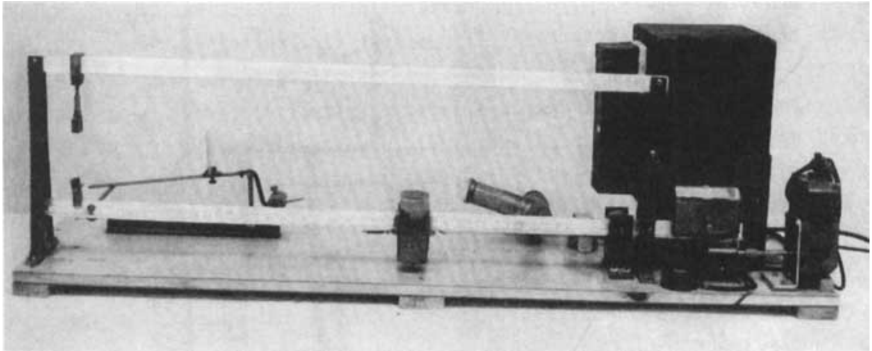


Fig. 3. Automatic tension testing apparatus specifically designed for the tension tests, simultaneously recording the tensile force and the elongation of the specimen.

With these aims in mind, a device has been designed and built, the principle of which is shown by the diagram in Fig. 2. In likeness with the previously mentioned devices employing a constant weight, the device contains a weigh-beam A supported at D so that it has two arms. At one end, the beam carries the counterweight B, and the loaded carriage C slides on the beam. The carriage is kept in uniform motion by the electric motor E, which also rotates the recording drum F. Each position of the carriage will thus be represented by a given vertical on the recording strip. The tendon to be examined is placed between the clamps H and I, of which H is adjustable in height. The recording is accomplished by optical means. A light beam emerging from a linear slit in front of the lamp J is reflected by the mirror K through a cylindrical lens L onto the light-sensitive paper on the drum F, on which a curve M is thus traced. Any elongation of the specimen causes the position of the beam A to change, and the light beam is reflected at a different angle from the mirror K. The tracing will show a corresponding vertical displacement. (Fig. 3.)

5.2 Behaviour of adhesions under effect of a static force

5.21 Method of investigation

The procedure in the tests for elucidation of the phenomena occurring when established tendon adhesions are subjected to a tension force was as follows.

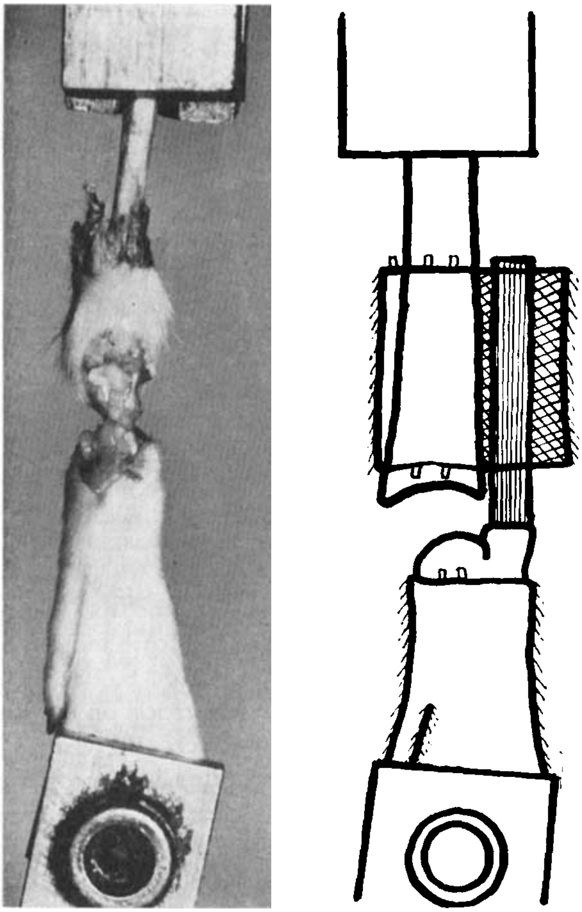


Fig. 4. Lower extremity specimen placed in the tension testing apparatus. The foot is attached to the lower leg only by the adhesions around the Achilles tendon. These circumstances are more clearly shown by the schematic diagram.

A careful examination was made of the operation wounds of the animals operated, traumatized and treated in previously stated manner (p. 27) for the tension tests. In some instances signs of infection were macroscopically observable: the wound had opened and there was purulent secretion. Such animals were excluded from the series.

Immediately after the animals had been sacrificed, their both hind legs were amputated at a point close to the knee in the proximal part of the lower leg. Exactly 1.5 cm from the point of attachment of the Achilles tendon to the calcaneus, the skin and underlying tendon together with the soft tissues were severed and a transversal incision was made down to the bone. All soft tissues proximal to this point were removed, leaving only the tibia. The talo-crural joint was exposed through an incision made on the anterior side of the ankle and continued around the calcaneus along a path distal to the point of attachment of the Achilles tendon. The ligaments of the talo-crural joint, all tendons, blood vessels and nerves passing over this point were cut. In this manner the Achilles tendon alone remained as a connection between lower leg and foot. On a length of 1.5 cm, the Achilles tendon and the lower part of the tibia were surrounded by a sleeve, consisting of the skin and soft tissues which had been left intact. In the tension tests the tendon was then pulled out from this sleeve.

For the test, the end of the tibia fragment was fixed in the upper clamp of the apparatus, which had been specially designed to admit the bone fragment. In some instances the end of the fragment was crushed when the clamp was tightened, resulting in loss of the specimen. The lower clamp was applied around the foot. The grip of the clamps was positive and no slip likely to introduce an error was observable. (Fig. 4.)

Upon completion of the clamping operation, and after the weighted carriage of the apparatus had been brought into its zero position, the tension testing apparatus was started. In the course of the test, the apparatus produced automatically a record on photographic paper, i.e., a curve showing the relationship between stress (magnitude of the load) and strain (elongation).

5.22 Interpretation of the tension test record

The character of the curves obtained by the action of the automatic recording device in the tension tests shall be described in the following.

The curve can be understood as a graph showing the force applied to the specimen and its elongation as functions of each other. The distance along the horizontal axis corresponds to the magnitude of the force and the distance along the vertical axis, to the elongation. The graph has a general shape as shown in Fig. 5.

At the start of the test a small amount of yielding occurs while the tendon becomes taut. After this, the graph ascends at a slope differing very little from the horizontal, at low values of the elongation. This part of the graph is linear, indicating that the elongation increased in proportion with the force. So far, Hooke's law of elasticity is completely valid in the test.

At the point consistent with a force F_1 equalling approximately one-fourth of the ultimate tensile strength (F_3), abrupt yielding occurred, in the course of which the elongation increased from λ_1 to λ_2 with hardly any increase of the applied force. A sudden stop of the yielding signifies that the tendon has become completely detached from its adhesions to bone and ligaments. After this point, from the force F_2 onward, follows another linear portion of the graph, with proportional increments of elongation and force. At a certain force F_3, to which corresponds the elongation λ_3, the tendon at last comes free of the surrounding skin and soft tissues. This value of the force is the tensile strength of the adhesions that have been established around the tendon, and λ_3 is the corresponding ultimate elongation.

Individual variations between specimens were noted in the value of F_1 and particularly in the amount of intermediate yielding $\lambda_2 - \lambda_1$. In some instances this yielding took place in two instalments. Some preparations were lost from the viewpoint of the present study for the reason that the record started at a point too high up on the paper and the ultimate portion ran off the paper.

For calibration of the tension testing apparatus, one end of the weigh-beam of an analytical balance was linked to the upper specimen clamp and the balance was weighted in steps of 50 g. The vertical scale of the graphs was calibrated in terms of elongation by shifting the position of the same clamp in steps of 1 mm with the aid of a micrometer screw and cathetometer. The tracings obtained by this means on the recording paper showed both scales to be linear in terms of the respective quantity, and the values read from the records are thus proportional to the actual force and elongation without any corrections. If the absolute values are desired, the F readings (in millimetres) have to be multiplied by 7.70 to obtain the force in grammes, while the factor 0.1665 converts the λ readings (in millimetres) to the corresponding elongations in millimetres.

5.3 Tension tests after 5 days

5.31 Experimental groups

The tension test series with specimens from animals sacrificed five days after the operation comprises 43 male rats. The animals were four months old and their average weight was 230 g. The operations by which their Achilles tendons were traumatized were performed by one and the same person in the previously described manner (p. 27). The operations on all animals of the series were carried out at one session. Immediately upon completed operation, the animals were divided into four groups as follows:

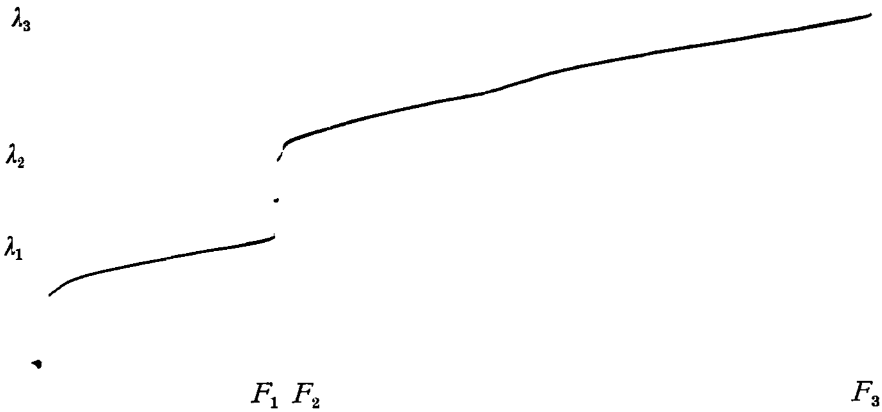


Fig. 5. Record obtained in a test with the tension testing apparatus. See text for detailed account.

(I) Control Group (C) — 11 animals. These animals received a daily injection of sodium chloride.

(II) Somatotropin Group (STH) — 11 animals. Daily injections or somatotropin were administered to these animals according to the schedule presented in the foregoing (p. 30).

(III) Thyrotropin Group (TTH) — 10 animals. Daily thyrotropin injections were administered in this group (see p. 30).

(IV) Somatotropin Plus Thyrotropin Group (STH + TTH) — 11 animals. The animals received a combined somatotropin and thyrotropin injection every day (see p. 30).

All animals concerned here were sacrificed five days after the operation and specimens derived from their both Achilles tendons were subjected to the tension test.

5.32 Forces required to detach the adhesions

The recorded value F_1 represents the force at which the first yielding occurred when the tendon became detached from its bony and ligamentary adhesions. As a rule, this point on the graph was easy to determine accurately. In the tests with some of the specimens the curve shows merely a slight angulation at this point.

The recorded value F_2 corresponds to the force prevailing at the moment when the yielding of the specimen has come to an end. This is the moment when the soft tissue adhesions come under tension. Since the preceding phase was one of yielding, the F_2 values are little different from the respective F_1.

The value F_3 indicates the force which is high enough to cause complete detachment of the tendon from its adhesions. This point, too, can be accurately determined because the recorded curve breaks off here. In some instances this end point of the curve fell outside the recording paper and the specimens were consequently lost. As can be seen from Figs. 6 and 8, the F_3 value is roughly four times the corresponding F_1.

Detachment from firm adhesions (F_1). The values in Fig. 6 reveal that the forces required to detach the tendon from its adhesions to bone and ligaments five days after the operation are not significantly different in the various hormone treatment groups from those found for the controls, nor mutually between the hormone treatment groups.

Yielding (F_2). The yielding phenomenon after the first detachment of the tendon proceeds virtually without incremental force. Consequently, the F_2 values are not essentially different from the F_1 values, nor can any significant differences be noted between the different groups. (Fig. 7.)

Tensile strength (F_3). The means stated in Fig. 8 show that the force required to set the tendon entirely free from its adhesions has undergone the following changes in the different hormone treatment groups:

— in the somatotropin group the strongest adhesions were noted after five days, and the force needed for their detachment was highest in this group;

— the force was next highest in the control group;

— in the thyrotropin group a smaller force sufficed to liberate the tendon from its adhesions than in the control group;

— in the somatotropin plus thyrotropin group the required force was smallest of all.

Statistical study with the aid of Student's *t* test revealed significant differences between the groups as follows:

The forces required in the STH + TTH group and in the TTH group are both smaller than that in the STH group at a highly significant level, and the difference by which the force in the STH + TTH group is smaller than in the C group is statistically significant. No other significant differences exist between the groups.

5.33 Elongations

The recorded value λ_1 represents the elongation at the moment when the tendon becomes detached from its firm adhesions. In absolute values, the λ_1 elongations are in the order of 2.5 mm. It is seen that a comparatively firm tissue is concerned.

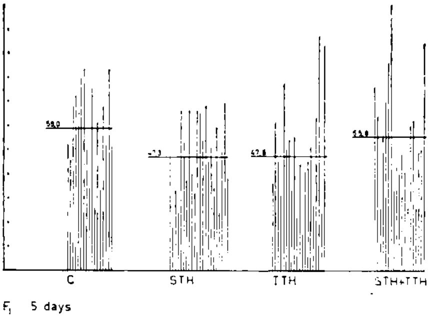
The recorded value λ_2 corresponds to elongation at termination of the yielding phase, subsequent to detachment of the bony and ligamentary adhesions. This value can be determined with good accuracy since the direction of the curve changes quite abruptly at the point in question.

The λ_3 value indicates the total elongation of the specimen immediately before the tendon is ultimately freed from its adhesions. Constituting the terminal point of the curve, this value was easily determinable from the records.

Detachment from firm adhesions (λ_1). The bony and ligamentary adhesions undergo only a small elongation prior to detachment. The differences between groups are also quite small and no statistically significant differences between the hormone treatment groups mutually or between them and the control group could be noted. (Fig. 9.)

Yielding (λ_2). The actual amount of yield is stated by the difference $\lambda_2 - \lambda_1$. It was noted in the foregoing that the λ_1 values are closely equal and instead of this difference we may therefore consider the λ_2 values. The means stated in Fig. 10 show that the elongation attendant on yielding of the tissue was smaller in the somatotropin group than in the control group, which was approximately equalled by the thyrotropin group in this respect. The greatest elongation was noted in the somatotropin plus thyrotropin group.

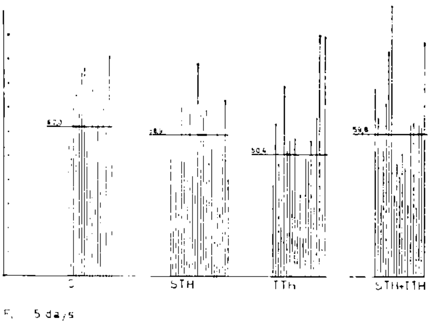
Ultimate elongation (λ_3). The elongation attained at the moment when the tendon became entirely free was smaller in the somatotropin group than in the control group, while its order of magnitude in the thyrotropin group was the same as in the controls. In the somatotropin plus thyrotropin group a higher ultimate elongation than in any other group was noted. (Fig. 11.)



Group	Mean	S.D.	Var.	Difference, compared to
C	59	18	0.30	C
STH	47	16	0.33	STH
TTH	48	22	0.45	TTH
STH +TTH	56	23	0.39	

*** Statistically highly significant
** Statistically significant
* Statistically almost significant

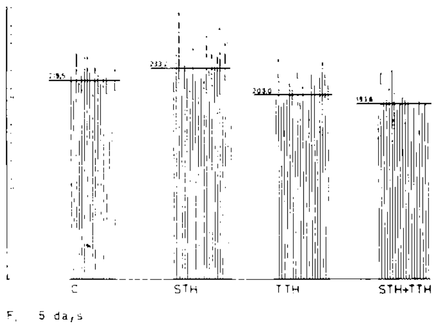
Fig. 6. Individual values (diagram on the left) and means (table on the right) of the force (F_1) required to detach the tendon from its firm adhesions, after five days.



Group	Mean	S.D.	Var.	Difference, compared to
C	62	18	0.30	C
STH	59	20	0.33	STH
TTH	50	22	0.44	TTH
STH +TTH	60	23	0.38	

*** Statistically highly significant
** Statistically significant
* Statistically almost significant

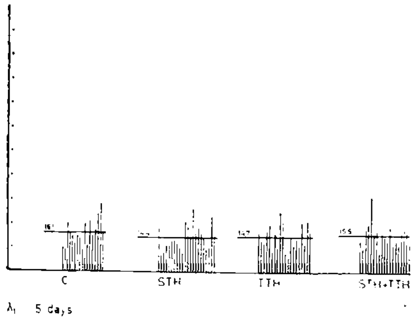
Fig. 7. Magnitude of the force (F_2) after yielding has taken place, after five days. Individual values and means as in Fig. 6. — F_2 is not greatly different from F_1 (Fig. 6).



Group	Mean	S.D.	Var.	Difference, compared to
C	220	29	0.13	C
STH	233	26	0.11	STH
TTH	203	27	0.13	*** TTH
STH +TTH	194	21	0.11	** ***

*** Statistically highly significant
** Statistically significant
* Statistically almost significant

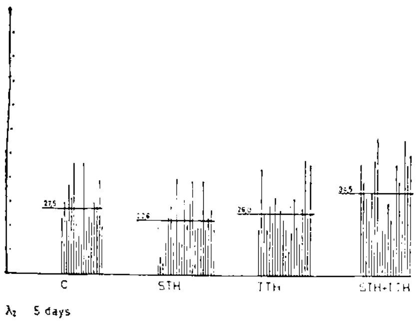
Fig. 8. Ultimate tensile strength (F_3) of the tendon adhesions after five days, shown as in Figs. 6 and 7, but with smaller ordinate scale. — The greatest force has been required in the STH group and the smallest force in the STH+TTH group. The statistically significant differences between groups found on analysis have been indicated at the intersections of horizontals and verticals in the table.



Group	Mean	S.D.	Var.	Difference, compared to
C	16	5	0.32	C
STH	14	5	0.35	STH
TTH	15	5	0.34	TTH
STH +TTH	16	5	0.32	

*** Statistically highly significant
** Statistically significant
* Statistically almost significant

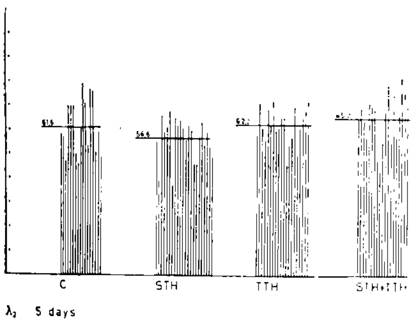
Fig. 9. Elongation under the force detaching the tendon from its firm adhesions (λ_1), after five days. No great differences between groups are observed.



Group	Mean	S.D.	Var.	Difference, compared to
C	28	10	0.35	C
STH	23	9	0.39	STH
TTH	26	10	0.39	TTH
STH +TTH	35	14	0.40	

*** Statistically highly significant
** Statistically significant
* Statistically almost significant

Fig. 10. Elongation after yielding has taken place (λ_2), after five days. The smallest elongation was obtained in the STH group.



Group	Mean	S.D.	Var.	Difference, compared to
C	62	10	0.17	C
STH	57	8	0.13	STH
TTH	62	7	0.11	TTH
STH +TTH	65	11	0.17	

*** Statistically highly significant
** Statistically significant
* Statistically almost significant

Fig. 11. Elongation under a force equivalent to ultimate tensile strength (λ_3), after five days. No statistically significant differences between groups.

5.4 Tension tests after 14 days

5.41 Experimental groups

The tension test series with specimens derived from animals sacrificed 14 days after the operation comprises 41 male rats of four months' age. Despite their equal age, the animals varied in weight between 210 and 320 g; the average weight was 255 g. The Achilles tendons of the rats were traumatized in the manner described before (p. 27). One and the same person performed all operations at one session. The animals were divided into the following four groups immediately upon operation, each group being placed in its particular cage:

(I) Control Croup (C) — 10 animals. The animals of this group were given daily injections of sodium chloride.

(II) Somatotropin Group (STH) — 10 animals. Daily injections of somatotropin (see p. 30) were administered in this group.

(III) Thyrotropin Group (TTH) — 10 animals. The animals received daily injections of thyrotropin (see p. 30).

(IV) Somatotropin Plus Thyrotropin Group (STH + TTH) — 11 animals. Combined somatotropin and thyrotropin injection was administered once every day (see p. 30).

The rats were sacrificed 14 days after the operation and their Achilles tendons were detached from their adhesions in the tension testing apparatus.

5.42 Forces required to detach the adhesions

As in the test series with animals sacrified five days after the operation, the recorded value F_1 represents the force which is able to detach the tendon from its bony and ligamentary adhesions. The yielding subsequent to this detachment came to an intermediate stop in some specimens, starting again after a slight increase of the force. Two values were obtained in these instances for F_1.

On the basis of the point at which the interrupted graph resumes its uniform course, the force F_2 prevailing at the end of the yielding phase can be determined. The yielding of one specimen from the control group took place in two stages.

The reading F_3 consistent with the terminal point of the recorded curve gives the force at which the tendon was completely detached from its adhesions. The automatic operation and recording of the tension testing apparatus and its design ensure that the accuracy of these readings is equally good as that of the values found in tests with smaller load.

Detachment from firm adhesions (F_1). The values seen in Fig. 12 display considerable scattering. There are no statistically significant differences between the hormone treatment groups mutually or between them and the control group.

Yielding (F_2). In likeness with the values of the force considered just before (F_1), the F_2 values are scattered over a fairly wide range. The increase of the force from F_1 to F_2 is insignificant, and no statistical analysis was thought to be worth while in respect of these observations. (Fig. 13.)

Ultimate tensile strength (F_3). The forces causing ultimate liberation of the tendon in the series referring to 14 days' hormone treatment (Fig. 14) are clearly different in the different groups, namely:

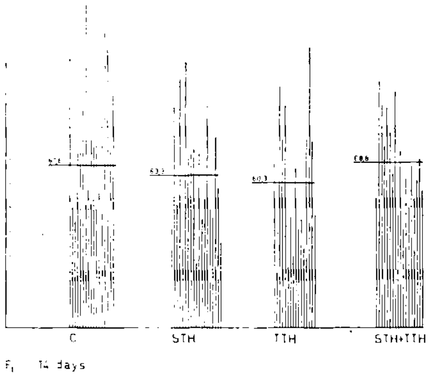
- in the somatotropin plus thyrotropin group the highest force was required to effect complete detachment;
- the force needed in the thyrotropin group was smaller, but it was distinctly superior to those in the other two groups;
- the next highest force was recorded in the control group;
- in the somatotropin group the tendon became completely detached with the smallest force.

According to statistical analysis by Student's t test, *the superiority of the force in the STH + TTH group over those in the C and STH groups is highly significant, and the difference against the TTH group is statistically significant. The force in the latter group is also higher than that in the STH group at a statistically significant level.* No other statistically significant differences with respect to the controls exist than that stated above.

5.43 Elongations

The λ_1 elongation corresponding to the moment of detachment from the firm adhesions consisted of two components in the case of three control specimens because the yielding ceased and continued again after some increase of the force.

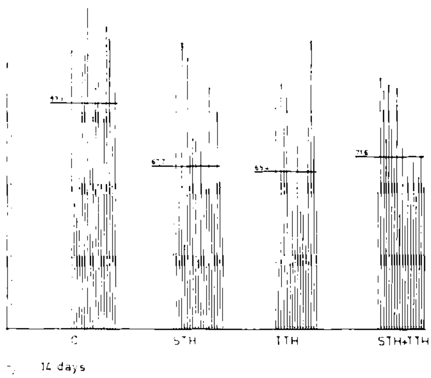
The elongation λ_2 at the end of the yielding phase was mostly easy to determine, except for one control specimen, in which another yielding phase ensued.



Group	Mean	S.D.	Var.	Difference, compared to C	STH	TTH
C	68	34	0.51			
STH	63	26	0.42			
TTH	60	26	0.43			
STH +TTH	69	18	0.25			

*** Statistically highly significant
** Statistically significant
* Statistically almost significant

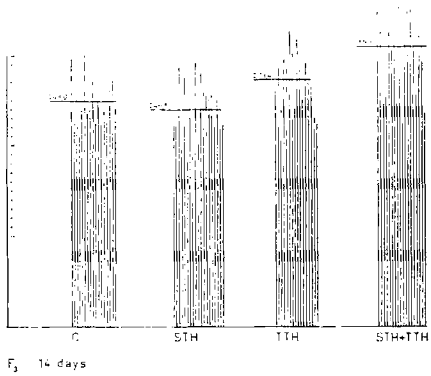
Fig. 12. Forces (F_1) required to detach the tendon from its firm adhesions, after 14 days. No significant differences between groups.



Group	Mean	S.D.	Var.	Difference, compared to C	STH	TTH
C	93	30	0.32			
STH	68	26	0.38			
TTH	65	29	0.44			
STH +TTH	72	19	0.26			

*** Statistically highly significant
** Statistically significant
* Statistically almost significant

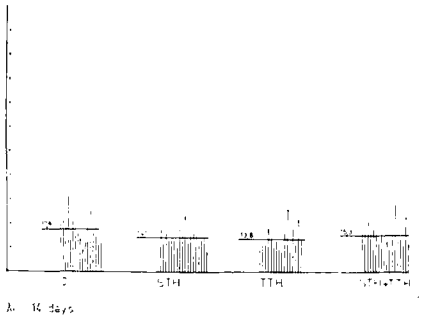
Fig. 13. Magnitude of the force (F_2) after yielding has taken place, after 14 days.



Group	Mean	S.D.	Var.	Difference, compared to C	STH	TTH
C	249	29	0.12			
STH	240	29	0.12			
TTH	273	20	0.07	*	**	
STH +TTH	309	41	0.13	***	***	**

*** Statistically highly significant
** Statistically significant
* Statistically almost significant

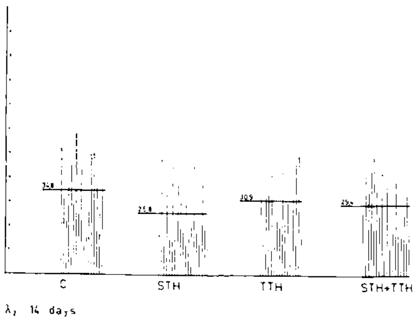
Fig. 14. Ultimate tensile strength (F_3) of the tendon adhesions, after 14 days; smaller ordinate scale than in Figs. 12 and 13. Statistically significant differences between groups are noted. In contrast to the results recorded after five days, the highest force has been required to detach the adhesions in the STH + TTH group and the smallest force in the STH group.



Group	Mean	S.D.	Var.	Difference, compared to		
C	18	6	0.33	C		
STH	14	3	0.23		STH	
TTH	14	5	0.36			TTH
STH +TTH	15	5	0.33			

*** Statistically highly significant
** Statistically significant
* Statistically almost significant

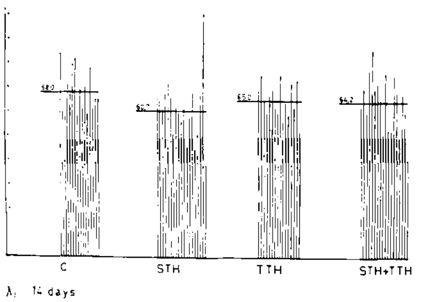
Fig. 15. Elongation (λ_1) under the force detaching the tendon from its firm adhesions, after 14 days.



Group	Mean	S.D.	Var.	Difference, compared to		
C	35	12	0.37	C		
STH	26	13	0.49		STH	
TTH	31	13	0.43			TTH
STH +TTH	29	12	0.41			

*** Statistically highly significant
** Statistically significant
* Statistically almost significant

Fig. 16. Elongation after yielding has taken place (λ_2), after 14 days.



Group	Mean	S.D.	Var.	Difference, compared to		
C	68	9	0.13	C		
STH	61	13	0.21		STH	
TTH	65	7	0.10			TTH
STH +TTH	64	11	0.17			

*** Statistically highly significant
** Statistically significant
* Statistically almost significant

Fig. 17. Elongation under a force equivalent to ultimate tensile strength (λ_3), after 14 days.

No difficulties were encountered in determining the ultimate elongation λ_3 except in a few instances, in which the curve extended past the upper edge of the recording chart.

Detachment from firm adhesions (λ_1). The values found for λ_1 are not essentially different in the hormone treatment groups mutually, nor from the value in the control group. No statistically significant differences can be expected to exist. (Fig. 15.)

Yielding (λ_2). The smallest amount of yielding is noted to have taken place in the somatotropin group. The other groups are little different from the control group in this respect. (Fig. 16.)

Ultimate elongation (λ_3). The ultimate values reached by the elongation at the moment of complete detachment of the tendon are not essentially different between the hormone treatment groups nor between these and the control group. The smallest value was found for the somatotropin group, but there is no statistically significant difference in comparison with the controls. (Fig. 17.)

5.5 Comparison of results after 5 and 14 days

5.51 *Forces required to detach the adhesions*

Detachment from firm adhesions (F_1). Comparison of the F_1 values found as means for the different groups five days and 14 days after the operation reveals that the force required to detach the tendon from its

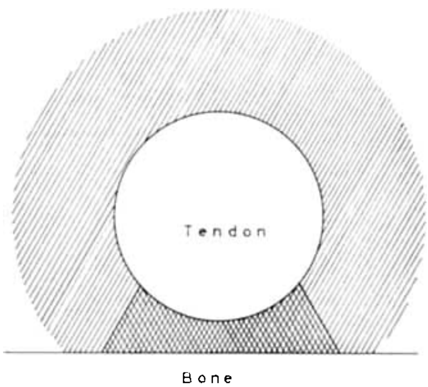


Fig. 18. Schematic diagram illustrating the fact that only a small part of the adhesions formed around the tendon attach the tendon to the underlying bone.

bony and ligamental adhesions increased in all groups during the intervening period. The graphical plot representing this change is a line of very nearly identical slope in each group, indicating that the increase is proportionally the same in all hormone treatment groups as well as in the control group. The increment is quite small, though, in all groups. It is to be concluded that hormone treatment has no effect on it. (Fig. 19.)

Referring to the schematic drawing presented in Fig. 18, it can be conjectured that there is only space for a small quantity of granulation tissue to be formed around the tendon on its side close to the bone. The bone would thus act in the manner of an interposition, preventing the formation of adhesions. Owing to this mechanical inhibition of the proliferation of granulation tissue the effect of time and of hormonal factors does not manifest itself.

Yielding (F_2). Since the yielding phenomenon requires virtually no incremental force, the five-day and 14-day F_2 values are not greatly different either, nor are there any statiscally significant differences. (Fig. 20.)

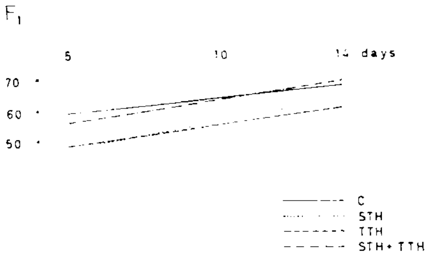
Ultimate tensile strength (F_3). More interesting observations are made on comparison of the forces needed to liberate the tendon entirely from its adhesions.

In the series of animals sacrificed five days after the operation, the highest force was required in the STH group to detach the tendon. The control group was next, followed by the TTH group, and the force was least in the STH + TTH group. In the 14-day series the sequence of the different test groups is exactly inverted: the required force is highest in the STH + TTH group, next highest in the TTH group and after that in the control group, while the least amount of force was needed in the STH group.

The graphical plots of the values in question reveal that the smaller the force required at five days after the operation, the greater is the slope at which the graph rises towards the value at 14 days. The increase is thus rather minimal in the STH group but highly marked in the STH + TTH group. All four lines intersect each other, forming a fairly regular fan. (Fig. 21.)

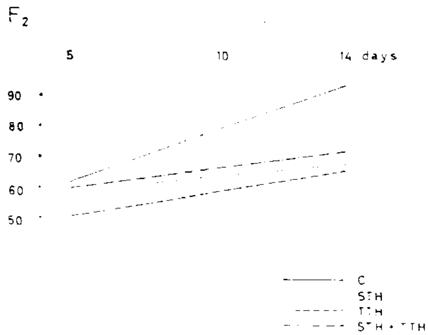
Analysis with the aid of Student's t test shows that statistically significant changes have ocurred in the ultimate tensile strength between five and 14 days after the operation:

The increase of ultimate tensile strength from five to 14 days is statistically highly significant in the STH + TTH and TTH groups and significant in the C group, whereas no statistically significant change is noted in the STH group.



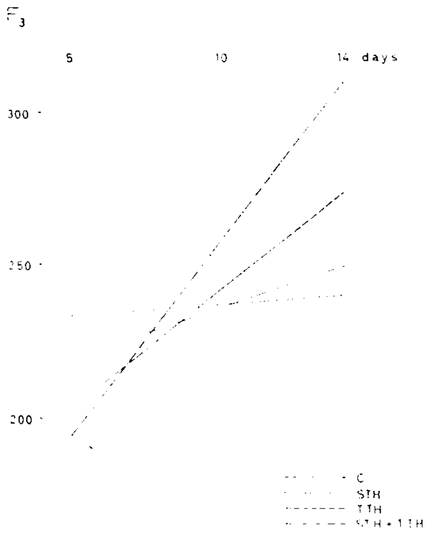
F_1	Group C	STH	TTH	STH +TTH
5 days	59	47	48	56
14 days	68	63	60	69
Change	9	16	12	13

Fig. 19. Comparison of the forces (F_1) required to detach the tendon from its firm adhesions after 5 and 14 days. No great differences are observed between groups.



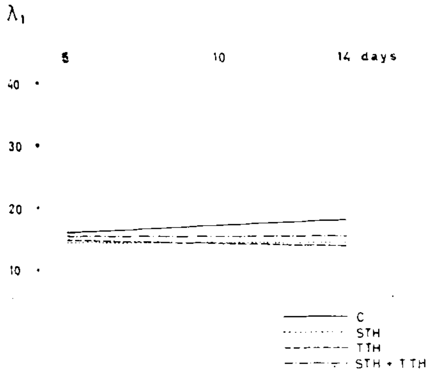
F_2	Group C	STH	TTH	STH +TTH
5 days	62	59	50	60
14 days	93	68	65	72
Change	31	9	15	12

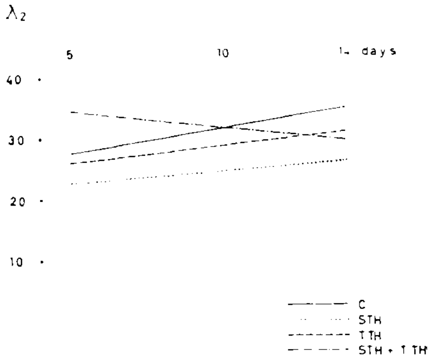
Fig. 20. Comparison of the forces recorded after yielding has taken place (F_2) after 5 and 14 days. The values are little different from those presented in Fig. 19.



F_3	Group C	STH	TTH	STH +TTH
5 days	220	233	203	194
14 days	249	240	273	309
Change	29**	7	70***	115***

Fig. 21. Comparison of the ultimate tensile strengths (F_3) after 5 and 14 days, revealing statistically highly significant increase of the force in the STH + TTH and TTH groups and no significant increase in the STH group.





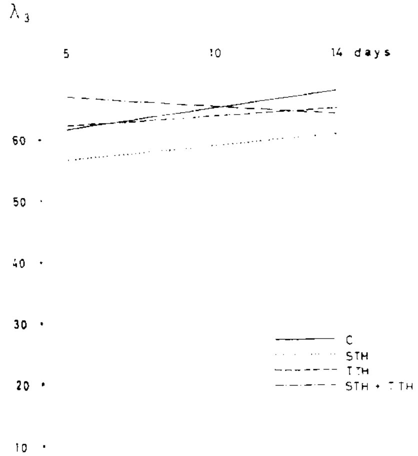


Fig. 22. Showing that the elongations (λ_1, λ_2, and λ_3) alone are poorly descriptive of the properties of the tissue. No noteworthy differences are observed between the elongations after 5 and 14 days.

Group	λ_1		λ_2		λ_3	
	5 days	14 days	5 days	14 days	5 days	14 days
C	16	18	28	35	62	68
STH	14	14	23	26	57	61
TTH	15	14	26	31	62	65
STH +TTH	16	15	34	29	65	64

No statistically significant changes.

5.52 *Elongations*

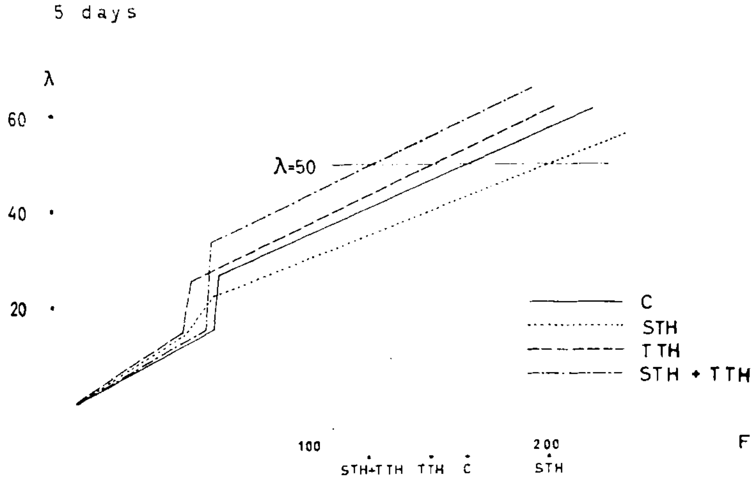
Comparison of the elongations at detachment of the firm adhesions, after yielding of the tendon and at the moment of ultimate liberation (λ_1, λ_2 and λ_3, respectively) yields no interesting observations, nor are the mutual magnitudes of their five-day and 14-day values such that statistical study of the differences would be worth while. As regards the adhesions between tendon and bone, this is thought to be due to the progressive character of the adhesion formation. At the yielding stage no additional energy is needed and therefore no differences in the structure of the tissue are elicited by the results. The elongation alone is only one of the quantities involved in the relationship implied by Hooke's law, and it is a poorly relevant one from the viewpoint of the physical properties of the substance which are of interest in this study. (Fig. 22.)

5.6 Relationship of elongation and force

In Fig. 23 the pairs of mutually consistent λ and F values found as means for the different test groups and referring to five and 14 days after the operation, respectively, have been plotted and joined by broken lines producing a schematic approximation of the curve characterizing the relationship between elongation and tensile force in each instance.

The diagram containing the graphs constructed in this manner to illustrate conditions at five days reveals that the graph of the STH + TTH group mostly runs on a higher level than all others and stops after a shorter horizontal course. The STH graph is lowest in its relevant portion and covers the longest horizontal distance. In the first-mentioned group the elongation is highest and the ultimate tensile strength is lowest, and the opposite is true for the latter. Among the graphs reflecting the behaviour at 14 days, that of the STH + TTH group is lowest in position and extends farthest horizontally; that is, the elongation is smallest and the ultimate tensile strength is highest in this group.

More lucid and informative comparisons can be made if a given fixed value is chosen for one of the two variables and the corresponding values of the other variable in the different instances are studied. Thus, we may choose for the elongation the fixed value $\lambda = 50$ (corresponding to 8.33 mm actual elongation), which has been indicated in Fig. 23. The corresponding, approximate F values will then be those stated in Table 3 on p. 92.



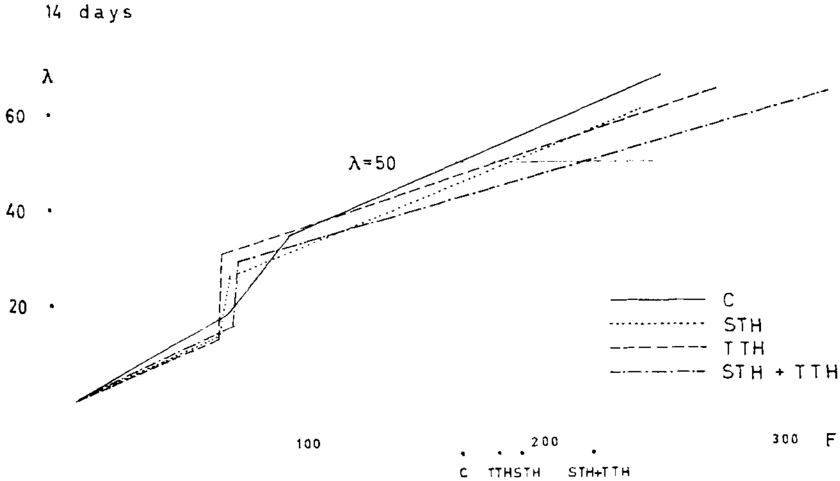


Fig. 23. Schematic force vs. elongation diagrams derived from the means of the forces (F_1, F_2 and F_3) and of the corresponding elongations (λ_1, λ_2, and λ_3), referring to tests after 5 and 14 days. After five days, the graph of the STH + TTH group is shortest and runs at highest level, while according to the tests after 14 days the graph of the same group is longest and lower than any other graph.

If Hooke's law $\lambda = Fl/EA$, or $F = EA\lambda/l$, is formally applied with the figures just considered, three of the variables involved, namely λ, l and F are given. The length of the adhesions $l = 5$ mm was determined by the traumatizing procedure, λ was fixed at a value equivalent to 8.33 mm, and F was read from the graphs. For constant A, the force F is then seen to be proportional to the modulus of elasticity E, which is a characteristic determined by the structure of the tissue subjected to tension. E is greater accordingly as the tissue is more compact and resistant to tension. The symbol A stands for the cross section area of the adhesions. If the values of A are separately determined, E can be calculated in each instance and inferences may be drawn from its value concerning the structure of the granulation tissue that has been formed.

5.7 Comments

It has been shown that traumatizing interference with the surface of the tendon, consisting of its compression without cutting any fibres of the tendon, was sufficient tó provoke formation of adhesions. It is to be concluded that care should be taken in clinical work not to touch the tendon with angular, hard instruments (cf. BUNNELL 1921, LITTLER 1947, MASON 1955, ALLEN 1955, VERDAN 1951).

In the present study the surface of the tendon and the surrounding tissue were subjected to trauma. Since adhesions could be produced in this manner, the theories and studies are corroborated according to which the healing of a tendon takes place by proliferation of external connective tissue (WEHNER 1923, HUECK 1923, NÄRVI 1926, SKOOG and PERSSON 1954, DAVIDSSON 1956, ASHLEY et al. 1960, POTENZA 1962).

The extremity was not immobilized subsequent to the operation, but this did not prevent adhesions from being formed. Irritation due to movement has been found to increase the reaction at the traumatized point and the adhesion formation (MASON and ALLEN 1941). This refers to the first two weeks, which is a period identical with the time covered by the present experiments.

Numerous clinical details are seen to be involved even though the best attempt was made to render the requisite tendon trauma in the experiments as uniform as possible in every case. Compression trauma of the kind described here is one of the commonest clinical tendon lesions. It is surprising that in spite of this fact exact experimental studies concerning this question are few in comparison with the ample clinical literature.

In view of the circumstance that the formation of adhesions on the side of the tendon against the bone is largely inhibited, the finding is readily understandable that there are no considerable differences between the different treatment groups in the magnitude of the force required to detach the *firm adhesions*. For the same reason the said force is not greatly different in the series referring to 5 days and 14 days after the trauma. Indications to the same effect can be derived from the behaviour of the elongation in the different test groups in both series. At all events, the slightly higher values at 14 days suggest the presence of a progressive process.

The *yielding* takes place without necessity of incremental force. The highest yield in the five-day series was noted in the STH + TTH group. This suggests that the granulation tissue may have been of looser structure in this group than in the other instances. The point shall not be discussed here, since obviously additional evidence is needed. The differences tend to level out towards the time of 14 days, which might be taken as an indication of homogenization ocurring in the structure of the tissues in the different groups. Such an assumption is well consistent with existing experience concerning the manner of healing of wounds and the general mode of action of hormones (Dunphy, Englebert and Udupa 1955, Jackson 1958, Williams 1955).

For the *ultimate tensile strength* the highest value in the five-day series was observed in the STH group, with a statistically highly significant difference against the STH + TTH and TTH groups. The same result has been reported by Saikku (1956) in his tension tests with cutaneous scars of the rat, although no statistically significant differences were elicited in his studies. The findings are in support of Li's (1960) statement that the rat is able to respond to somatotropin from a foreign species. In respect of the cause responsible for the changes in tensile strength of the granulation tissue produced under hormonal influence reference can be made to the studies of Amromin (1950) and Cavallero (1953), who found that somatotropin stimulated the growth of granulation tissue.

Paradoxically, the highest ultimate tensile strength in the 14-day series was encountered in the STH + TTH group, which differed at a statistically highly significant level from the STH and C groups in this respect. The question naturally arises why the STH group should present the highest tensile strength at five days and the lowest at 14 days and why the opposite is true for the STH + TTH group. This must be connected with changes in the quality or quantity of adhesion tissue. Changes in quality are suggested by Scow's (1951) studies and changes in the quantity of granulation tissue by those of Taubenhaus (1950).

Further light is thrown on this question by comparison of the five-day and 14-day tensile strengths in the different groups. It is seen that in the STH group no worthwhile change takes place during this time. It would seem that granulation tissue under the effect of somatotropin loses its proliferative ability at an earlier stage than that in the controls. In the STH + TTH and TTH groups the differences between the five-day and 14-day values are statistically highly significant and it is concluded that a lively process must have been in progress (cf. EDWARDS and DUNPHY 1958). Indications to the same effect are furnished by the elongation values.

5.8 Synopsis of the tension tests

1. In the tension tests the recorded curve representing the relationship between elongation and tensile force was found to be composed of three parts, which correspond to the following phases: detachment of the firm adhesions; yielding; and detachment of the soft tissue adhesions. *The force required to overcome the adhesions to soft tissues was about four times that needed to detach the firm adhesions.*

2. The administration of hormones had no effect on the tensile strength consistent with detachment of the firm adhesions in either test series (after 5 days and after 14 days).

3. The hormones exerted no noteworthy effect on the amount of yielding in either test series.

4. No great differences between the groups with different hormonal treatment were elicited in respect of elongation in either test series.

5. a) *The ultimate tensile strength at the end of the productive phase (after 5 days) was highest in the somatotropin group, with the other groups following in the order: control, thyrotropin, and somatotropin plus thyrotropin group. Statistically highly significant differences were observed between the STH group on one hand and the TTH and STH + TTH groups on the other hand, and between the control and STH + TTH groups.*

b) *The ultimate tensile strength in the collagen phase (after 14 days) was highest with combined somatotropin and thyrotropin treatment; this group was followed by the thyrotropin, control and somatotropin groups, in this order. Statistically highly significant differences existed between the STH + TTH group on one hand and the control and STH groups on the other hand, while the differences between the TTH group on one hand and the STH + TTH and STH groups on the other hand were statistically significant.*

6. *Comparison of the results obtained in the test series carried out after 5 days and after 14 days revealed the greatest increase in ultimate tensile strength in the somatotropin plus thyrotropin group, the difference between the values relating to 5 days and 14 days being statistically highly significant, as was the difference by which the ultimate tensile strength increased from 5 to 14 days in the thyrotropin group. The value increased during this period also in the control and somatotropin groups, the increment in the first-mentioned group amounting to a statistically significant difference.*

6. PLANIMETRIC STUDIES

6.1 Assessment of granulation tissue quantity

6.11 Quantitative methods

The commonest method employed in studying the effect of various factors on tissues is the gravimetric method. Changes of weight may result from change of the absolute tissue quantity but also from that of its physical-chemical properties, e.g. of the fluid content.

Simplicity is an advantage of the gravimetric method when a separate, easily detachable organ is concerned. Considerable difficulties are encountered on the other hand if, for instance, the factors affecting the amount of granulation tissue are to be investigated in this manner. It is then necessary to apply a special technique in order to separate the generated granulation tissue from adjacent tissues. Selye (1953) developed a special so-called granulation pocket method for this purpose. More generally used is the cotton knot embedded, as suggested by Stebbins and Stoerk (1954), as a foreign body among the tissues to cause irritation resulting in formation of granulation tissue. Viljanto and Kulonen (1962) used a piece of cellulose sponge to the same end.

Granulation tissue produced by foreign-body irritation acquires a specific structure characterized by giant cells. No such cells could be observed in the granulations formed about a tendon. Gravimetric methods could not be used with success in studying the adhesions formed around tendons.

Easy distinction of the granulation tissue formed around an injured tendon is only possible by histological means. Most of the histoquantitative methods are accordingly based on measurement of the intensity at which a given radiation is transmitted by the microscopic preparation or of the change in wavelength occurring at transmission. The determinations are usually relative and refer only to a given part of the microscopic preparation. It may suffice to mention briefly some of the methods based on this principle.

The absorption of light outside the visible spectrum when it penetrates the microscopic preparation may be determined (Barer 1950). Microradiography may be used to determine the absorption of very soft x-rays; the absorption has been found to depend on the dry mass of the tissue (Engström and Lindström 1950). The electron microscope may also be used in identical manner (Scott 1943). The property of autofluorescence of tissues is applicable in some instances (Pringsheim 1949). Phase contrast and interference microscopes provide opportunities for quantitative refractometry (Barer, Ross and Tkaczyk 1953). Eränkö (1955) has presented several photometric methods in his book.

6.12 Planimetric methods

The force and elongation were studied in the preceding chapter in respect of their mutual relationship. Such investigations are most naturally complemented by data on *cross section area*. Considering the adhesions around a traumatized tendon in particular, their cross section area can be determined by planimetric methods. The specimen is destroyed in the tension test involved in the above-mentioned, preceding study and the preparations for planimetric study cannot be made from the same individual specimens, unfortunately.

Determination of areas with a grid. When the eye-piece of the microscope has a grid composed of small squares, the size of a given area can be found by immediate counting of the whole squares it covers and estimation of the parts of squares cut by its boundary. The same method of assessment can also be applied if an image of the preparation is projected on a screen divided into squares.

Determination of areas with the planimeter. Planimeters are mechanical devices operating on a mathematical principle and giving direct readings which represent the area of a plane figure with quite high accuracy. It is particularly advantageous to use a planimeter when various parts of one and the same figure have to be measured. The planimeter then also gives their aggregate area without need of addition.

Determination of areas by planimetric weighing. In determining the areas of simple, large figures such as those concerned when the cross section areas of adhesions around a tendon have to be assessed, the method of planimetric weighing has proved to be most appropriate. It means that an image of the preparation is projected on a sheet of homogeneous paper of uniform thickness, after which the boundaries of the area to be determined are traced on the paper, a corresponding piece is cut out and its weight is determined with an analytical balance. All cut-outs representing samples of one and the same experimental series may be weighed in aggregate and the result divided by the number of preparations to find the mean value with great accuracy. The planimetric weighing method is widely used in quantitative histology. TALA (1952) may be mentioned among those who have employed it.

The range of auxiliary techniques employed in aid of planimetry may be enlarged to include quantitative histochemistry and medical chemistry, but it does not seem appropriate to review the numerous possibilities which then present themselves.

6.2 Experimental animals and groups

The principles outlined in the foregoing (p. 26) were observed in selecting and treating the experimental animals, which were male, white Wistar rats in all experiments concerned here. The entire material comprised 222 animals. All endeavours were made to render the conditions and the care of the experimental animals equal to those in the preceding test series in order that all systematic factors affecting the wound healing might be excluded.

The operative interference and traumatization of the rats' tendons were carried out as described previously (p. 27). This procedure for traumatization of the rat's Achilles tendon had already proved adequate, and the method was best conducive to uniformity of the local factors influencing the healing process.

Likewise, the postoperative treatment of the material took place in accordance with the previously described principles, the experimental animals being immediately divided into four groups here, too. The groups were thus:

(I) Control Goup (C) — 63 animals altogether;
(II) Somatotropin Group (STH) — 51 animals altogether;
(III) Thyrotropin Group (TTH) — 53 animals altogether;
(IV) Somatotropin Plus Thyrotropin Group (STH + TTH) — 55 animals altogether.

The medication applied in each group was the same as described on p. 30. Each group was subdivided into two subgroups, the animals of which were sacrified five days and 14 days after the operation, respectively.

6.3 Methods

After the animals subjected to operation, traumatization and treatment in the stated manner had been sacrificed, their operation wounds were examined. All individuals displaying microscopically observable signs of infection were discarded.

Trauma had been caused in the tendons on a portion 0.5 cm in length, at 1 cm distance from the point of attachment of the Achilles tendon to the calcaneus. The tendon was severed in the proximal portion of its traumatized part, exactly 1.5 cm from its calcaneus insertion. This was done by incising transversally the skin covering the tendon, the tendon itself and all soft tissues down to the bone. Another similar transverse incision was then made immediately above the calcaneus. Exercising great care, the tendon, soft parts and skin were detached as a single block from the bone along the periosteum. In this manner a specimen was obtained which comprised the traumatized portion of the tendon, the surrounding tissues and the skin covering them. The proximal end was marked for later identification by means of a silk thread sewn into it prior to fixation.

With the aim in mind that the stain should distinctly reveal all the different components of connective tissue, *F.S.A. fixation* (80 ml distilled water, 20 ml formol, 4 g mercuric chloride and 5 ml acetic acid) was employed. The stain chosen in this study was *Pentachrome II* (Movat 1955), which demonstrated all structural components of the connective tissue in one and the same section. This five-colour staining method involves the following constituents:

(1) Alcian Blue — imparts a blue or blue-green stain to the ground substance and to its acid mucopolysaccharides;

(2) Weigert-Hart's Resorcin-Fuchsin — imparts a dark purple stain to the elastic fibrils;

(3) Weigert's Iron-Haematoxylin — imparts a black stain to the nuclei;

(4) Orange-G — stains the cytoplasm yellow or orange-yellow;

(5) Diphenyl Fast Red — imparts a bright red stain to the collagen.

The Pentachrome II procedure enabled the granulation tissue that had been formed to be clearly distinguished from its surrounding tissues.

Upon completed fixation, the specimen could be suspended from the thread that had been sewn into it and embedded in paraffin in vertical position. This served to make sure that the specimens would be in correct position and the microscopic cuts would be made of the traumatized

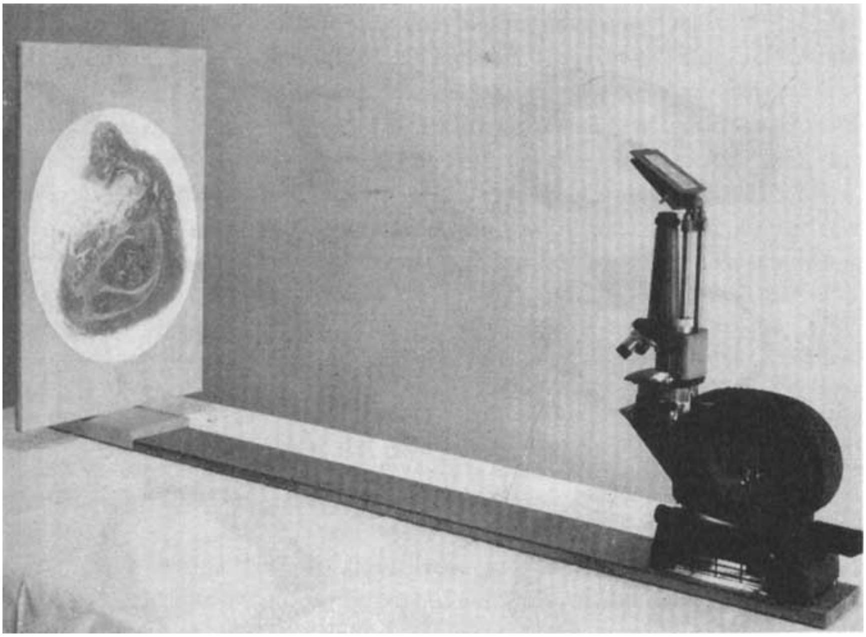


Fig. 24. Application of the Leitz microprojector for planimetric determination of tissue cross section areas.

portion proper. Only such preparations were accepted for study in respect of which it was subsequently evident from the cross section surface of the histological section that the cut had been made at right angles to the tendon. If microscopic examination revealed that the cut deviated from the direction perpendicular to the traumatized portion of the tendon, the preparation was not included in the study. Some other preparations had also to be discarded which had been damaged at sectioning. In some of the instances an acceptable preparation could be obtained by repeating the sectioning and staining.

The microscopic preparations were placed in a simple Leitz microprojector (magnification about 60×) and their magnified image was projected on a sheet of paper. The contours of the granulation tissue that had been formed around the tissue could then be traced on the paper. (Fig. 24.)

The use of standard writing paper in the planimetric procedure described on p. 59 was excluded for the reason that it displays considerable variations in weight. Instead, a special brand of superfine, highly homogeneous

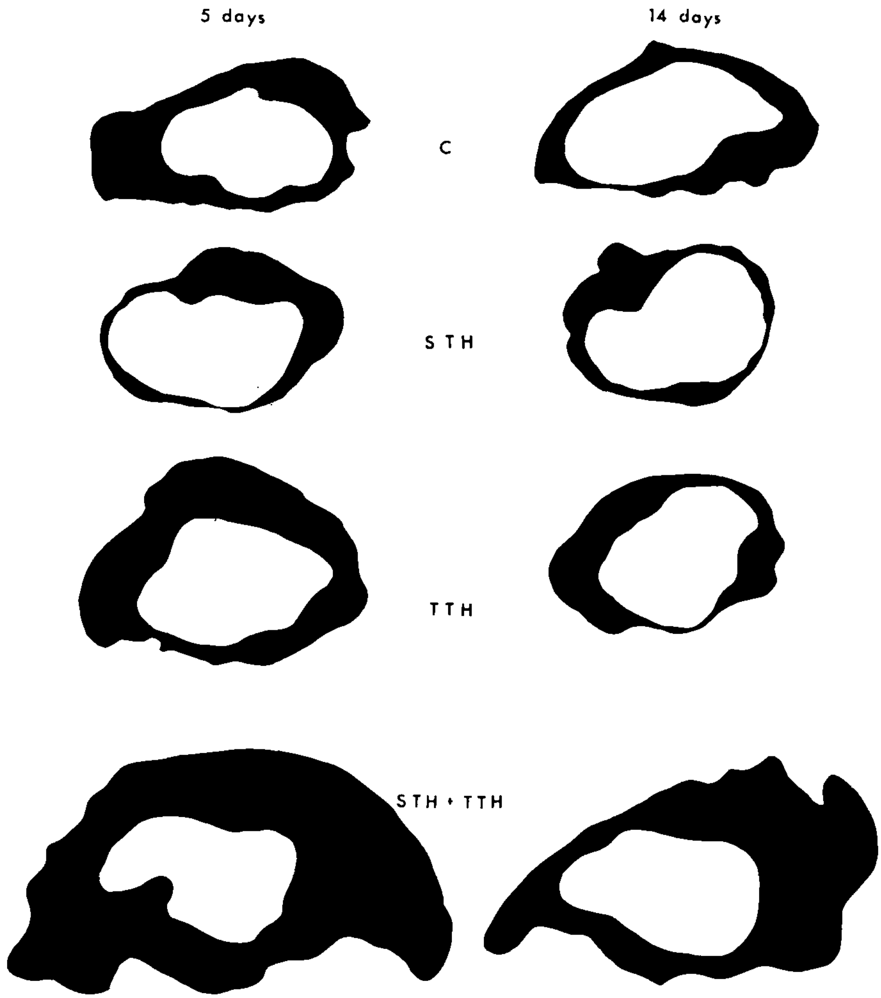


Fig. 25. Example showing adhesion cross sections found in the different test groups after 5 and 14 days. The areas are smallest in the STH group and largest in the STH + TTH group with synergistic action of both hormones, after five days as well as after 14 days.

paper manufactured by the mill Kankaan Paperitehdas was used. The paper was made on special order without water-mark and had a weight of 60 g per m^2, the manufacturers guaranteeing its weight differences not to exceed 4 %.

The granulation tissue was organized around the tendons in such manner that there was a very thin course on the surface against the bone, whereas the thickest granulation layer was found on the surface against the skin, where the scar after the operation was located. The conditions are illustrated by the schematic diagram in Fig. 25 and by Figs. 26–27.

The areas to be determined were cut out from the paper sheets and weighed with an analytical balance providing an accuracy of 0.1 mg, which was found to be sufficient. All weighings were performed at one time, at a given temperature and relative humidity of the air in order to exclude any effects that might be caused by varying moisture content of the paper.

The results of the weighings, in mg, are relative values representing the cross section areas. In the following they shall be stated and used as such. When desired, the true area of each cross section in mm² is found by multiplying the relative value by 0.00474. This conversion factor was determined from the average weight of ten separate paper cut-outs obtained in the same manner when the microscopic preparation had been replaced by a Bürker counting cell with one-tenth-millimetre grating, each cut-out corresponding to 1 mm².

The data yielded by the described, modified planimetric procedure can be brought into relationship with the results of the tension tests on the theoretical basis that the ultimate force required to rupture a homogeneous specimen equals the product of its tensile strength and its cross section area.

6.4 Planimetric studies after 5 days

6.41 Experimental groups

The material was the same as that used in the tissue analysis (p. 76). Since, however, only completely undamaged preparations could be subjected to the planimetric procedure, part of the preparations included in the tissue analyses had to be discarded here.

The part of the material concerning animals sacrificed five days after the operation comprises altogether 117 rats. The operative treatment, medication and methods of investigation were the same as described in the foregoing (pp. 27, 30). As before, the animals were divided into four groups:

(I) Control Group (C) — 33 animals;
(II) Somatotropin Group (STH) — 28 animals;
(III) Thyrotropin Group (TTH) — 27 animals;
(IV) Somatotropin Plus Thyrotropin Group (STH + TTH) — 29 animals.

— Control group (C).

— STH group. There is little granulation tissue around the tendon, but in the original cut it is clearly distinct owing to its intense red staining.

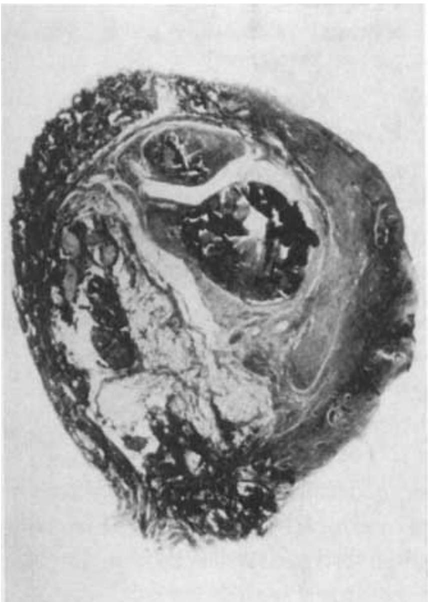
— TTH group. A great amount of granulation tissue of loose appearance has been produced.

— STH + TTH group (synergistic action of the hormones). The extraordinarily massive granulation formation threatens to rupture the scar.

Fig. 26. Cross sections of Achilles tendons after five days. The tendon and its adhesions are seen at the top. At the bottom the skin is open after removal of the bone. The scar after the operation can be seen in the upper right corner.

– Control group (C).

– STH group. The adhesions are small in area.



– TTH group. The traumatization did not extend to the boundary surface between the different parts of the tendon; the epitenon is therefore untraumatized at this point and no adhesions have formed.

– STH + TTH group (synergistic action of the hormones). It can be seen that the huge amount of granulation tissue is not limited to the area around the tendon: the scar of the skin is also notably thickened.

Fig. 27. Cross sections of Achilles tendons after 14 days. The skin has healed and collagen is seen in ample amount in all instances.

Although both extremities could not be included in the study in the case of all animals, owing to the above-mentioned causes, the entire usable material was evaluated without exercising any selection. The series are therefore comparatively extensive, which is advantageous in the interest of reliable results.

6.42 Cross section area of tissue components

The results obtained on planimetric study with the animals sacrificed five days after the operation can be found in Table 1 and are also shown by the graphs in Fig. 28, separately for each of the four above-mentioned groups. The numerical values refer to the weights of the paper cut-outs in mg (cf. p. 59), which are mutually comparable.

The results reveal that the smallest cross section area of the granulation formed about the tendon occurred in the STH group. Next in order of magnitude follow the C and TTH groups. The granulation area was largest in the STH + TTH group, that is, with the animals which had been given combined somatotropin and thyrotropin treatment.

Statistical analysis by Student's *t* test, applied to the different groups in all possible combinations of two, revealed *statistically highly significant differences between all groups except between the C and TTH groups.*

The observation can thus be made that combined somatotropin and thyrotropin medication produced substantially superior adhesions as regards their cross section area. No conclusive difference is seen between the control and thyrotropin groups, but both are distinctly inferior to the STH + TTH group. In the somatotropin group the adhesion cross section was smallest of all and it was clearly less than in any other group.

6.5 Planimetric studies after 14 days

6.51 Experimental groups

The planimetric studies on specimens derived from rats sacrificed 14 days after the operation cover a material of altogether 105 rats. The operative treatment, medication and methods of investigation have been described in the foregoing (pp. 27, 30). The same material, with some further animals added, is also concerned in the subsequently reported tissue analysis studies (cf. p. 63). The results to be stated below therefore lend themselves to comparisons with the histological findings (p. 76).

Table 1. Comparison of adhesion cross section areas found after 5 and 14 days in the different groups by planimetric study. Statistically significant differences between groups and significant changes from 5 to 14 days are noted. (Levels of statistical significance indicated for differences between groups at the intersections of horizontals and verticals in the table, for changes from 5 to 14 days in the squares on the central diagonal).

	5 days								14 days			
	Var.	S.D.	Mean	Group		STH ↓	TTH ↓	STH +TTH ↓ , Difference compared to				
5 days	0.25	104	414	C	**	*		***	C	324	86	0.27
5 days	0.32	98	303	STH	***		*	***	STH	276	87	0.32
5 days	0.31	144	465	TTH	*	***	***	***	TTH	328	80	0.24
5 days	0.23	188	817	STH +TTH	***	***	***	***	STH +TTH	654	229	0.35
	Difference, compared to				↑ C	↑ STH	↑ TTH		Group	Mean	S.D.	Var.

Squares on diagonal between both halves of the table: Difference 5 days – 14 days

Unit: mg of paper cut-out weight

*** Statistically highly significant
** Statistically significant
* Statistically almost significant

As before, four groups of experimental animals have been considered:

(I) Control Group (C) — 30 animals;
(II) Somatotropin Group (STH) — 23 animals;
(III) Thyrotropin Group (TTH) — 26 animals;
(IV) Somatotropin Plus Thyrotropin Group (STH + TTH) — 26 animals.

6.52 Cross section area of tissue components

The results obtained on planimetric study with the animals sacrificed 14 days after the operation have been presented in Table 1. The ultimately sought values could be recorded for both extremities in part of the material only, and it was not considered appropriate to make a distinction between the results relating to the Achilles tendon of the left foot and of the right foot. Instead, all results have been treated as a compound series.

The results reveal that the smallest cross section area of the adhesions that developed around the tendon occurred in the somatotropin group, with the C and TTH groups next. The area was largest in the group of animals under combined somatotropin and thyrotropin medication,

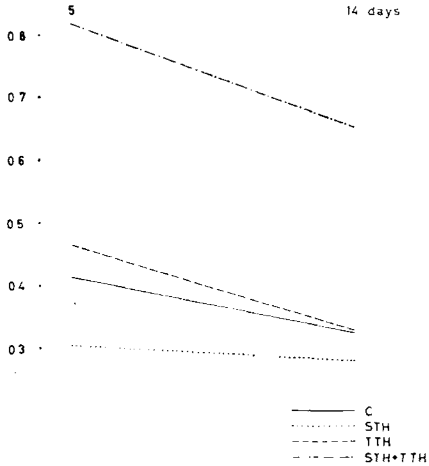


Fig. 28. Comparison of adhesion cross sections. The cross section area was largest in the STH + TTH group and smallest in the STH group. The latter group also displayed the smallest reduction in cross section area from 5 to 14 days.

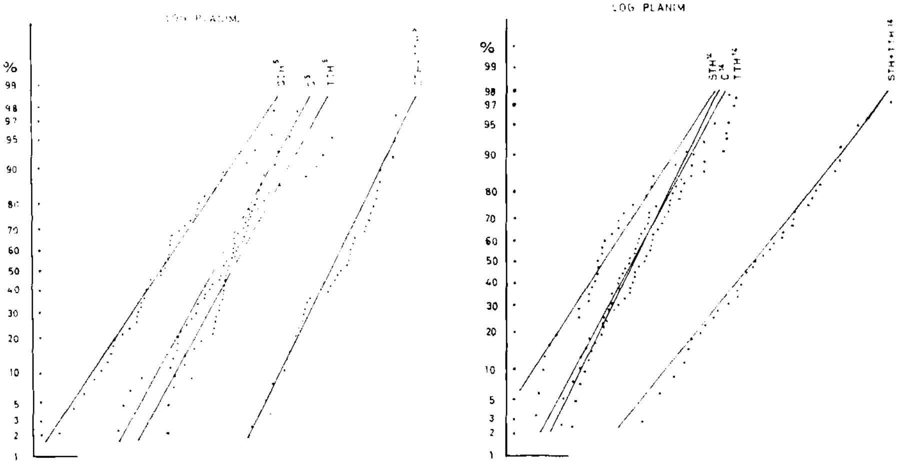


Fig. 29. Individual values (logarithmic) of adhesion cross section area in the different groups after 5 and 14 days, plotted as cumulative frequency curves on a probability chart. The plots are fairly well consistent with straight lines, indicating distributions close to normal, and mutually closely parallel, implying equal variances (S.D./Mean) in the different test series.

which differs essentially from the other groups. According to Student's *t* test there is a *statistically highly significant difference between the STH + TTH group and each of the other groups*. Between the C, STH and TTH groups, in their different combinations by two, at the most statistically almost significant differences can be noted.

6.6 Comparison of results after 5 and 14 days

Comparison of the values recorded at 5 and 14 days after the operation reveals that a distinct regression in cross section area of the granulation tissue around the tendon takes place in the intervening period in all groups. The mean cross section area was thus reduced by slightly more than 20 % in the C and STH + TTH groups, but only by almost 9 % in the STH group. Fig. 28 furnishes a concise idea of the essential findings.

The values found for the cross section area at 5 and at 14 days, respectively, display a *statistically significant difference in all but the STH group*, in which no significant difference was established (Table 1).

6.7 Statistical distribution of the cross section area

The logarithms of the individual cross section areas were plotted on a probability chart (i.e., their cumulative frequency graph was drawn) for each of the eight subgroups (5 and 14 days, respectively, and groups C, STH, TTH and STH + TTH) (Fig. 29). The plots fall on straight lines with statisfactory accuracy, indicating that the distribution is close to normal. It is also seen that the straight lines have largely the same slope, which is equivalent to nearly identical variances (quotient of S.D. and mean value; cf. Table 1). The intersection of the straight lines with the 50 % horizontal corresponds to the (geometric) mean in each subgroup, and these, too, agree fairly closely with the arithmetic means in Table 1.

6.8 Comments

The planimetric study of the granulation tissue formed around the tendon after traumatization was undertaken in order to obtain additional data supplementing the information furnished by the tension tests. The ideal would have been to achieve one-to-one correspondence between cross section area and ultimate strength. However, since the specimens were destroyed in the tension test, the planimetric study had to be made with a different set of specimens and conclusions can only be drawn from the corresponding mean values found for the different groups. Of the other conceivable approaches by which the quantity of granulation tissue might have been evaluated, the gravimetric method was not practicable, nor would its results be relevant since the ultimate force producing rupture of different kinds of tissue is not simply proportional to the weight of the tissues. Determination of the dry matter quantity, e.g. by means of absorption of radiation or by micro-incineration, might be able to throw further light on the object of study.

Under the effect of *somatotropin* the cross section area of the granulation tissue at five days and also at 14 days was found to be less than in the controls. In other words, no increase of the granulation tissue as reported by TAUBENHAUS (1950) and by TAUBENHAUS and AMROMIN (1950) could be established. Their studies, as also those of CAVALLERO (1953), had been made with infected tissue. WILLIAMSSON and NEUMAN (1954), failing to elicit the stimulating effect of somatotropin in every instance, claim that large doses exert an inhibitory influence. The dosage employed in the present study, in combination with thyrotropin, did not cause any inhibition. However, opposite opinions are also encountered in the literature. In agreement with the present findings, SPAIN and MOLOMUT (1953) could not observe any increase in the quantity of granulation tissue under the effect of somatotropin. — The present results can be summarized to the effect that *the animals subjected to the effect of somatotropin had the smallest granulation cross section; furthermore, its change during the period from five to 14 days was smallest in their group.* The last-mentioned observation seems to indicate early completion of the granulations, that is, early maturation.

The effect of *thyrotropin,* either immediate or indirect (JEFFRIES 1949), was found to produce after five days a granulation tissue of greater cross section area, at a highly significant level, than in the somatotropin group. The granulation tissue quantity was also higher than in the controls. ASBOE-HANSEN (1959) states that the increase in mass is due to increase of the acid mucopolysaccharides of the ground substance. SAIKKU (1956), too, noted increased weight of the granulations under the effect of thyrotropin. In the present studies, the figures relating to 14 days do not show any noteworthy excess of the quantity of granulation tissue in the thyrotropin series over that of the controls. — The findings can be summarized to the effect that *thyrotropin increased the cross section area of the granulations around the tendon most strongly at the early, productive phase of granulation formation.*

In the group of animals treated with *somatotropin and thyrotropin in combination* more extensive adhesions, at a highly significant level, than in any other group were established at five days as well as 14 days. In his studies on the repair of experimental fractures, Koskinen (1959) obtained powerful osteogenesis by means of simultaneous somatotropin and thyrotropin administration, with large callus area as measured from x-rays and with achievement of clinical stability in half of the time needed in the controls. — Summarizing the present findings, it can be said that *simultaneous administration of somatotropin and thyrotropin caused the strongest increase in quantity of granulations, producing the largest adhesion cross sections at five days as well as at 14 days after the operation.*

In all hormonal treatment groups the cross section area of the granulation tissue was seen to decrease during the period between five and 14 days. This shrinkage of the scar tissue is a phenomenon which has been observed early in surgery and it is often undesirable. Attention was paid to it already by Carrel and DuNoüy (1921) in their studies. They report that the contraction commences suddenly at 5—7 days after the trauma. In the present study *the contraction noted in the somatotropin-treated animals was less than in any other group,* suggesting that smaller changes have taken place in the granulation tissue.

Collating the results obtained in the tension tests and those of the planimetric studies referring to the time 14 days after the operation, the observation can be made that the mean values of the ultimate force producing rupture are proportional to the mean adhesion cross section area. The highest ultimate force corresponds to the largest cross section area (in the STH + TTH group) and the smallest force was required when the cross section was smallest (in the STH group). No similar regularity could be established in respect of the observations made at five days. The planimetric study is not able to account for the differences in rupture limit between the different groups. It seems obvious that differences between the groups in structure of the tissue and their progressive change during the period between five and 14 days must be concerned. This aspect calls for further investigation.

6.9 Synopsis of the planimetric studies

1. In the test series *after 5 days* the following observations were made: *The cross section of the granulation tissue was smallest in the somatotropin group and larger in the control, thyrotropin and somatotropin plus thyrotropin groups, in this order. All differences between groups were statistically highly significant except between the control and thyrotropin groups.*

2. In the test series *after 14 days* the following observations were made: *The cross section of the granulation tissue was again smallest in the somatotropin group, which was followed by the control, thyrotropin and somatotropin plus thyrotropin groups, in this order. Statistically highly significant differences were only established between the somatotropin plus thyrotropin group, in which the cross section was largest, and all other groups.*

3. During the period *between 5 and 14 days a reduction in cross section area of the granulation tissue occurred in all groups. The decrease was minimal in the somatotropin group and did not amount to a statistically significant change.*

7. HISTOLOGICAL STUDIES

Histoquantitative methods. One of the earliest procedures of quantitative tissue analysis was the counting of blood cells. This implies a count of the number of cells in a compartment of given height, per unit area. In the preceding chapter a planimetric method has been described in which the area under investigation is divided into a number of squares by means of a superimposed screen. When this net of the screen has one hundred squares, the per cent composition of various tissues is easily determinable. In the science of quantitative histology, this procedure is known as *area sampling.* It has the drawback of being slow and cumbersome if a histologically complex tissue is concerned. Granulation tissue with its numerous different components is such a tissue.

Another method, known as *point sampling* (CHALKLEY 1943) also makes use of a similar screen, but the percentages of the different tissues are determined by the number of line intersections at which each of them is encountered. If the procedure is repeated after an arbitrary shift of the screen, a combination of the area and point sampling methods is obtained. All these methods have been described in detail in ERÄNKÖ's (1955) text-book.

The relative distribution of areas can also be determined altogether without determining actual surface areas. The principle according to which this can be done has long been used e.g. in the science of forest mensuration (ILVESSALO 1921) and in geology, and the procedure is known as line surveying. When the total area under examination is known, the absolute quantity of each kind of tissue can be calculated from the relative figures.

This *line sampling* method was applied and developed in quantitative histology by UOTILA (1940) and by UOTILA and KANNAS (1952). According to their procedure, a histological preparation is evaluated by drawing a straight line across the slide and determining in given, relative units the aggregate lengths of intersects of this line running across each type of tissue.

The line sampling method is excellently suited for determination of the proportions in which different cell groups occur in glandular tissues, and

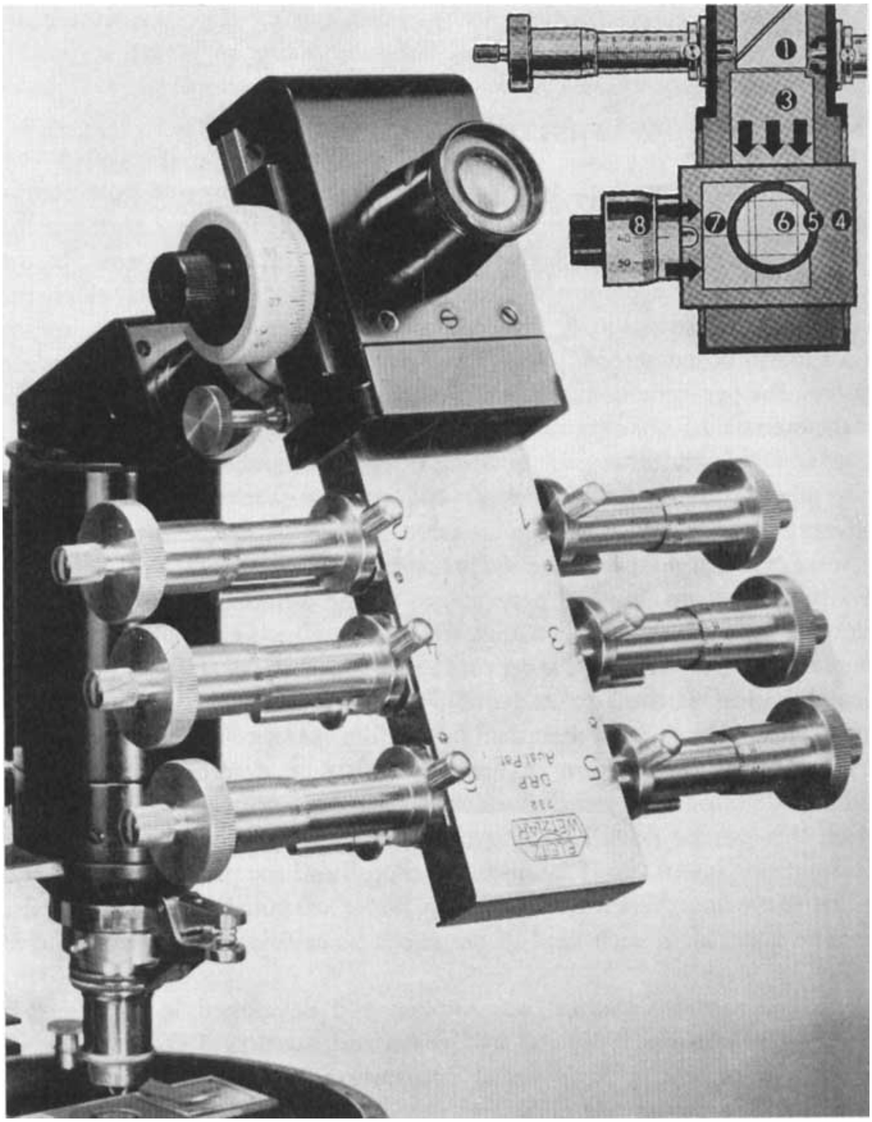


Fig. 30. The Leitz integrating microscope micrometer eye-piece.

it has been applied in histoquantitative studies on the thyroid gland by TALA (1952) and of the prostate by v. HELLENS (1955). The repair of experimental fractures has been studied with its aid by KOSKINEN (1959, 1965), and the behaviour of granulation tissue in particular was thus studied by SAIKKU (1956).

A convenient modification of the line sampling method involves the use of an *integrating eyepiece micrometer* (Fig. 30). The measurements are then performed in the focal plane of the eyepiece lens, by which a number of secondary sources of error will be eliminated. The integrating eye-piece manufactured by the LEITZ company has several micrometer drums, any one of which can be used to shift the crosshair, so that the transects of each kind seen along the sampling line can be accumulated individually to give immediate aggregate readings. The integrating eye-piece constitutes an excellent aid in microscopic-anatomical quantitative studies.

7.1 Experimental animals and groups

While in the studies on tensile strength the preparations were destroyed by rupture in the test, the specimens used in the planimetric studies were usable for histological tissue analysis as well. Furthermore, the latter does not necessitate completely intact cross cuts and consequently also such specimens could be evaluated which had to be discarded in the planimetric studies because they were partially broken. As a result, the material concerned here is somewhat larger in extent than that of the planimetric series, but individual comparison of both kinds of results specimen by specimen remains possible. The total number of operated animals was 271 and in most instances both hind legs could be examined.

The same subdivision into experimental groups was applied as in the other studies, namely:

(I) Control Group (C) — 81 animals;
(II) Somatotropin Group (STH) — 64 animals;
(III) Thyrotropin Group (TTH) — 63 animals;
(IV) Somatotropin Plus Thyrotropin Group (STH + TTH) — 63 animals.

The animals in each group received injections as described on p. 30. One set of subgroups was sacrificed after five days and another after 14 days.

7.2 Methods

After each animal had been sacrificed, the traumatized portion of the Achilles tendon was carefully excised including the skin and surrounding soft tissues down to the periosteum. Fixing was perfomed as has been described in the preceding chapter (p. 60), with F.S.A. solution. Upon embedding in paraffin, the tendons were sectioned transversally at 6 μ and the cuts were stained according to the Pentachrome II method.

The tissue analysis was carried out by observing in the microscope the linear quantities of different tissues, progressing radially outwards from the surface of the severed tendon. It was noted that the collagen fibres seem to have a tendency to arrange themselves tangentially close to the surface of the tendon, and the resulting source of error had to be taken into account (ERÄNKÖ 1955). The tissue quantities were recorded with the aid of an integrating eyepiece micrometer (by Ernst Leitz, Wetzlar, Germany). The principle is the same as that of the line sampling described by previous authors (UOTILA 1940, UOTILA and KANNAS 1952), except that the measurements were made in the eyepiece plane as opposite to moving the microscope stage. The present technique eliminates a number of secondary sources of error and is thus conducive to greater accuracy.

The eye-piece has two crossed lines, of which the vertical line is used to indicate the line along which the analysis is to proceed. It was positioned radially with respect to the tendon cross section and the analysis was commenced at the surface of the tendon. The horizontal hairline can be moved with the aid of any of six wedges, each wedge being actuated by its own micrometer drum. If the line is moved by turning always the particular drum assigned to the kind of tissue under the cross lines at the moment, the final readings of the drums after a given length of vertical has been traversed will give the relative quantities of six different kinds of tissue. Several sets of parallel measurements can be made if the vertical line is displaced laterally. By rendering easy and reliable the quantitative determination of physiological and pathological changes in tissue distribution, the integrating eyepiece micrometer constitutes a valuable aid in microscopic-anatomical investigation.

The following tissue components were chosen as those to be recorded in analyzing the granulation tissue with respect to its tissue distribution:

(a) *Collagen.* This tissue was readily distinguishable since it acquired a bright red stain in the Pentachrome II procedure.

(b) *Fibrocytes.* The nuclei in the connective tissue cells were stained black and their cytoplasm was stained a light yellow, and they were consequently easy to discern.

(c) *Capillaries.* The sampling line was always placed so that no other than newly formed blood vessels were traversed. When a blood vessel was encountered, its endothelial cell tissue was also included in the transect reading, in addition to the lumen.

(d) *Inflammation cells.* The number of such cells was low, and in some preparations none fell on the survey lines. These cells were easily distinguished by their fragmented nucleus.

(e) *Empty space.* The preparations showed also areas with no stainable matter. Obviously the ground substance had been washed away at such points.

(f) *Ground substance.* The ground substance was stained light-blue or bluish green and was thus clearly distinct from any other tissue components.

No giant cells were observed in the preparations, which indicate the presence of foreign-body irritation (SELYE 1953, STEBBINS and STOERK 1954, SAIKKU 1956, VILJANTO and KULONEN 1962). This indicates, together with the small amount of inflammation cells, that the results recorded here cannot have been detrimentally influenced by the presence of inflammation. Any preparations revealing leukocytes in a quantity exceeding 4 % were considered to be infected and were discarded.

In the interest of lucidity, in connection with the treatment of each particular group only the mean values are stated and the statistically significant differences, if any, between the different groups and subgroups are mentioned. The primary results *in toto* have been deposited in the Helsinki University Library, Helsinki, Finland.

7.3 Tissue analysis after 5 days

7.31 Experimental groups

The part of the material concerning animals sacrificed five days after the operation comprises altogether 141 rats. The number may be considered rather unnecessarily high, but it is to be viewed against the paradoxical results obtained in the tension tests and the endeavours to clarify their cause. It was not possible for one person to perform the operation on this many animals at one time. Since, therefore, the operations had to be carried out by consecutive groups, separate control series were established for each group and subjected to the same operation. Subsequent scrutiny showed that there are no noteworthy differences between the different control series. It was therefore considered justified to lump the different partial series of each kind together in view of more convenient handling of the data.

Combined in this manner, the experimental animals constitute the following groups:

(I) Control Group (C) — 41 animals;
(II) Somatotropin Group (STH) — 34 animals;
(III) Thyrotropin Group (TTH) — 33 animals;
(IV) Somatotropin Plus Thyrotropin Group (STH + TTH) — 33 animals.

7.32 Distribution of tissue components

Fibrocytes. No closer distinction is made in this study between the different types of connective tissue cells. This was not possible at the microscopic magnification employed in this work. Endeavours were merely made to distinguish normal cells from inflammation cells.

The results of the tissue analysis stating the amounts of fibrocyte tissue are seen in Fig. 31. The table in this figure reveals that connective tissue cells occur in greatest abundance in the thyrotropin and somatotropin plus thyrotropin groups, while they are less numerous in the somatotropin and control groups. The percentages show a *statistically significant difference of the TTH group from the STH + TTH group and a highly significant difference from the other two groups. A highly significant difference still exists between the STH + TTH and C groups.*

The observation can thus be made that in the specimens taken after five days fibrocytes occur in greater abundance in the hormone treatment group in which thyrotropin was administered.

Ground substance. The staining method employed in this work caused the ground substance to become blue or blue-green. It was the predominant tissue component in the preparations in all but the somatotropin group. Some error in determination of the ground substance may be introduced by the fact that some of the fibrocytes lost their nucleus at sectioning and their cytoplasm was then difficult to distinguish from the ground substance.

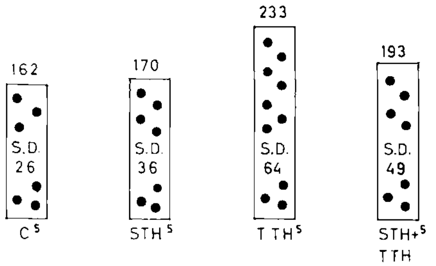
Fig. 32 and its table show the results obtained in determination of the relative ground substance quantity at five days. The quantity of ground substance was highest in the somatotropin plus thyrotropin group and least in the somatotropin group. Its relative quantity *in the STH + TTH group was higher than in the C and STH groups at a statistically highly significant level and significantly higher than in the TTH group, while the C group showed ground substance more than in the STH group at a highly significant level.*

Collagen. The relative quantity of red-staining collagen, too, was determined with the aid of the integrating eyepiece micrometer (cf. p. 75). Scrutiny of the preparations revealed that the collagen was not completely evenly distributed all over the section in the specimens from animals sacrificed after five days. It appears that the formation of collagen fibres started at certain centres of crystallization. Such centres are the spots where collagen existed even before, such as the tendon and the larger blood vessels. Thus, the first collagen fibres arrange themselves tangentially to the cross section of the tendon. For this reason the tissue analysis measurements were made along a line progressing radially outward from the surface of the tendon.

The results referring to the relative collagen quantity can be seen in Fig. 33 and its table. It can be seen from the table that collagen occurred at relatively highest quantity in the somatotropin group, the control group ranging next. Less collagen was seen in the granulation tissue in the thyrotropin group and least of all was recoıded in the adhesions in the somatotropin plus thyrotropin group. All values are clearly apart and it was found that *statistically highly significant differences exist between any two groups*. The observation is thus made that hormone administration exerts a distinct effect on the formation of collagen in granulation tissue.

Capillaries. In the figures reflecting the amount of new blood vessels in the granulation tissue, the areas covered by endothelial tissue and the lumen were included. If there were leukocytes within the lumen, they were not taken into account separately. In the choice of the sampling line such points were avoided where, for instance, the large blood vessels of the mesotenon would have been traversed.

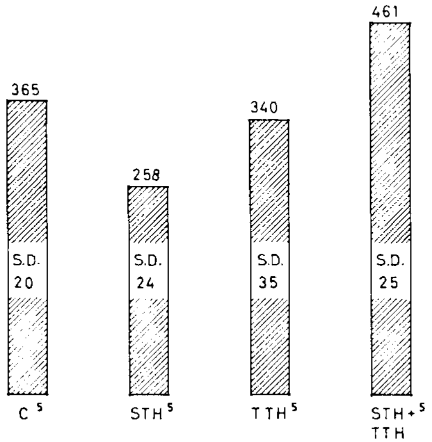
The relative contribution of new blood vessels to the analyzed tissue in the different groups can be seen from Fig. 34. The table in this figure reveals that the relative quantities of capillary space found in the different groups are not essentially different. This implies that the administration of hormones did not substantially affect the relative quantity of blood vessels formed in the granulation tissue.



Group	Mean	S.D.	Var.	Difference, compared to		
C	162	26	0.16	C		
STH	170	36	0.21		STH	
TTH	233	64	0.27	***	***	TTH
STH +TTH	193	49	0.25	***	***	**

*** Statistically highly significant
** Statistically significant
* Statistically almost significant

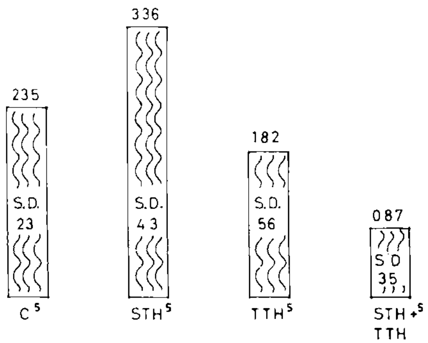
Fig. 31. Relative quantities of fibrocytes in the adhesions after five days. Number of cells greatest in the TTH group, differing from the other groups at a statistically significant level. (Mean values on top and inscribed S. D. values: Thousandths of mean transect length; likewise in the table.)



Group	Mean	S.D.	Var.	Difference, compared to		
C	365	20	0.05	C		
STH	258	24	0.10	***	STH	
TTH	340	35	0.10			TTH
STH +TTH	461	25	0.54	***	***	**

*** Statistically highly significant
** Statistically significant
* Statistically almost significant

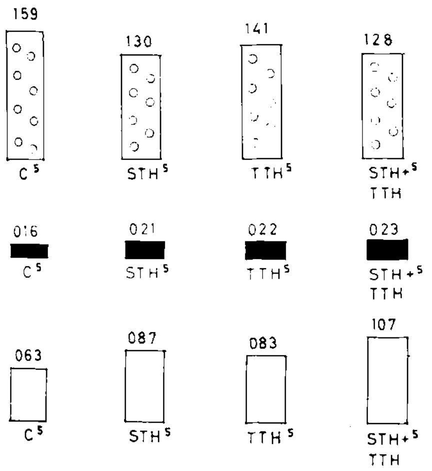
Fig. 32. Relative quantities of ground substance in the adhesions after five days. Greatest amount observed in the STH + TTH group, differing at a statistically significant level from the other groups, and least in the STH group. (Inscribed values as in Fig. 31.)



Group	Mean	S.D.	Var.	Difference, compared to		
C	235	23	0.09	C		
STH	336	43	0.13	***	STH	
TTH	182	56	0.31	***	***	TTH
STH +TTH	87	35	0.40	***	***	***

*** Statistically highly significant
** Statistically significant
* Statistically almost significant

Fig. 33. Relative quantities of collagen in the adhesions after five days. Greatest amount of collagen in the STH group, statistically highly significant differences between groups. (Inscribed values as in Fig. 31.)



5 days	Group C	STH	TTH	STH +TTH
Capillaries	159	130	141	128
Leukocytes	16	21	22	23
Empty space	63	87	83	107

No statistically significant differences between groups.

Fig. 34. Relative amounts (top to bottom) of capillaries, leukocytes and empty space in the adhesions after five days. No statistically significant differences. (Inscribed values as in Fig. 31.)

Leukocytes. The inflammation cells in the preparations are distinguishable by their smaller size and their fragmented nucleus. As a rule they were rather scanty, only a few cells per transect. Leukocytes seen in the lumen of traversed blood vessels were not taken into account. In the majority of the preparations the number of leukocytes was less than 4 %. In some specimens this figure was considerably exceeded, which were accordingly considered to be inflamed and were discarded. The last-mentioned preparations also contained separate leukocyte accumulations, in which bacteria could be observed. In many of these cases, the epithelium at the site of the wound had failed to close and it was encrusted, with bacteria present in great number.

The results can be seen in Fig. 34. The values found for the relative quantity of leukocytes are not essentially different. This implies that the administration of hormones did not substantially influence the number of leukocytes in the granulation tissue investigated.

Empty space. The sections contained some areas from which the stainable matter had been washed off in preparation. It seems obvious that this has been interstitial matter, seeing that cracks, which occur now and then as artefacts, are readily recognizable.

The occurence of empty spaces in the different groups is seen from Fig. 34. Empty spaces were seen to occur in somewhat greater abundance in the somatotropin plus thyrotropin group than in any other, but the differences are not very notable.

7.4 Tissue analysis after 14 days

7.41 Experimental groups

The material used in quantitative tissue analysis comprises altogether 130 animals sacrificed 14 days after the operation. It turned out to be a difficult task to prepare good cuts of the hard tendon tissue, and microscopic preparations were not obtained from all animals.

By combining the subgroups of animals with different operating dates, the following groups were obtained:

(I) Control Group (C) — 40 animals;
(II) Somatotropin Group (STH) — 30 animals;
(III) Thyrotropin Group (TTH) — 30 animals;
(IV) Somatotropin Plus Thyrotropin Group (STH + TTH) — 30 animals.

7.42 Distribution of tissue components

Fibrocytes. In the specimens taken at 14 days the fibrocytes are squeezed flat between the collagen fibres. Since the fibres have no consistent orientation, the tissue analysis takes part of the fibrocytes into account in their longitudinal extension.

The results can be seen in Fig. 35 and its table. The relative quantity of fibrocytes in the specimens taken at 14 days was found to be closely the same in all groups and there are no statistically significant differences.

Ground substance. Less ground substance occurred in the specimens taken at 14 days than in those from animals sacrificed five days after the operation. Between the strongly red-stained collagen fibres and close to them the ground substance occasionally assumed a brownish tinge, which may be merely due to reflection, seeing that elsewhere the ground substance was stained blue in normal manner.

The results can be seen in Fig. 36 and its table. At 14 days, as at five days, the relative quantity of ground substance was still highest in the somatotropin plus thyrotropin group and lowest in the somatotropin group. However, the differences have become considerably less and their statistical significance is lower in level than at five days. The quantity of ground substance is *greater at a statistically highly significant level in the STH + TTH and TTH groups than in the STH group. That in the STH +*

TTH group still exceeds significantly the quantity in the C and TTH groups, and the quantity in the C group is significantly higher than in the STH group.

Collagen. In the specimens taken at 14 days collagen was the predominant tissue component in all experimental groups, extending as a uniform network all over the preparation. As in the case of the animals sacrificed at five days, the quantitative determinations were made along a line radial to the tendon cross section. This renders the observations relating to five and to 14 days mutually comparable.

The occurrence of collagen 14 days after the operation can be seen from Fig. 37 and its table. At 14 days, too, the highest relative collagen quantity was seen in the somatotropin group. The differences between the other groups are small. There is no longer any statistically significant difference between the TTH and C groups. *The STH group still displays a statistically significant difference from the C group and highly significant differences from the other treatment groups.*

Capillaries. In determining the quantity of new blood vessels, the endothelial cell tissue was also included in addition to the lumen. In all other respects, too, the same principles were followed as in the analyses relating to five days of hormonal treatment.

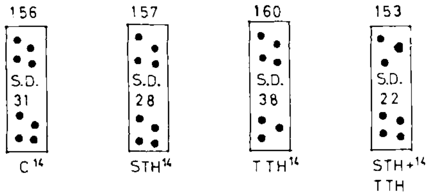
The results are shown in Fig. 38. It can be seen that the relative abundance of capillaries was not greatly different in the different groups. It is concluded that the administration of hormones did not essentially affect the formation of new blood vessels.

Leukocytes. The number of leukocytes at 14 days was reduced in every group from that recorded at five days. In most of the preparations no leukocytes at all were seen.

The relative quantity of leukocytes, which was very small in all groups, is seen from Fig. 38. No great differences can be noted between the different groups.

Empty space. Between the collagen fibres there occur areas from which all ground substance has been washed away. The area within the lumen of blood vessels was not included in the empty spaces.

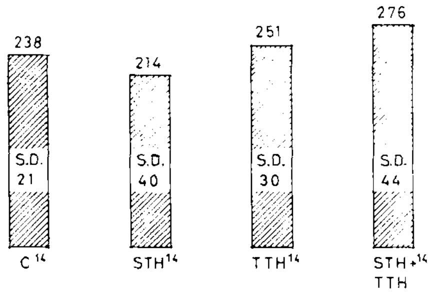
The relative quantities of empty space are shown by Fig. 38. Unstained, empty spaces occurred in equal relative quantity in the different groups. It can be concluded that the hormone administration had no effect on the relative amount of matter washed off.



Group	Mean	S.D.	Var.	Difference, compared to		
				C	STH	TTH
C	156	31	0.20			
STH	157	28	0.18			
TTH	160	38	0.24			
STH +TTH	153	22	0.14			

*** Statistically highly significant
** Statistically significant
* Statistically almost significant

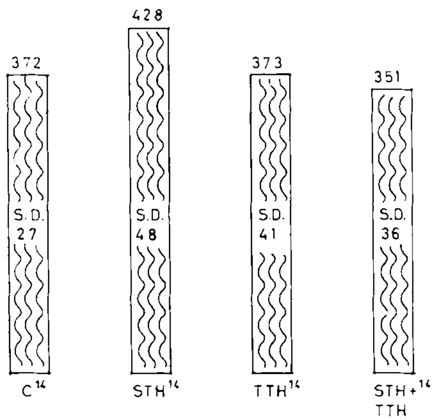
Fig. 35. Relative quantities of fibrocytes in the adhesions after 14 days. There are no longer any statistically significant differences. (Mean values on top and inscribed S. D. values: Thousandths of mean transect length; likewise in the table.)



Group	Mean	S.D.	Var.	Difference, compared to		
				C	STH	TTH
C	238	21	0.09			
STH	214	40	0.19	**		
TTH	251	30	0.12		***	
STH +TTH	276	44	0.16	**	***	**

*** Statistically highly significant
** Statistically significant
* Statistically almost significant

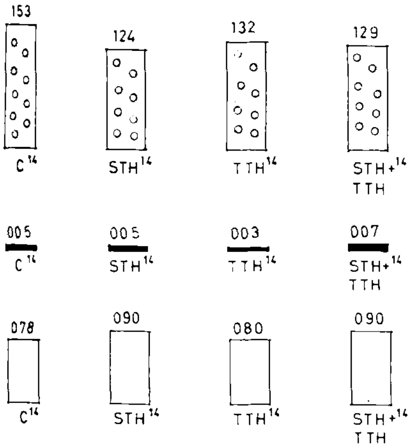
Fig. 36. Relative quantities of ground substance in the adhesions after 14 days. The amount is still highest in the STH + TTH group at a statistically significant level, but the differences are about to become equalized. (Inscribed values as in Fig. 35.)



Group	Mean	S.D.	Var.	Difference, compared to		
				C	STH	TTH
C	372	27	0.07			
STH	428	48	0.11	**		
TTH	373	41	0.11		***	
STH +TTH	351	36	0.10	***	***	**

*** Statistically highly significant
** Statistically significant
* Statistically almost significant

Fig. 37 Relative quantities of collagen in the adhesions after 14 days. The amount is still greatest at a statistically significant level in the STH group, but the differences are about to become equalized. (Inscribed values as in Fig. 35.)



14 days	Group C	STH	TTH	STH +TTH
Capillaries	153	124	132	129
Leukocytes	5	5	3	7
Empty space	78	90	80	90

No statistically significant differences between groups

Fig. 38. Relative amounts (top to bottom) of capillaries, leukocytes and empty space in the adhesions after 14 days, equal in the different groups. (Inscribed values as in Fig. 35.)

7.5 Comparison of results after 5 and 14 days

Comparison of the tissue analysis results reported in the foregoing reveals that remarkable changes have taken place in all hormone treatment groups during the period from five to 14 days in the per cent distribution reflecting the structure of the granulation tissue. The changes noted with regard to the most essential tissue components, namely, fibrocytes, ground substance and collagen are illustrated by Table 2.

It is seen that the relative collagen quantity increased in all groups, but decidedly most strongly in the STH + TTH group, while the increase was least in the STH group. The amount of fibrocytes is seen to have gone down; the effect may be partially due to flattening of the cells. The ground substance decreased consistently in all groups; its change, too, was strongest in the STH + TTH and least in the STH group.

The diagram in Fig. 39 gives a clear idea of the changes established by the tissue analysis measurements. The smallest changes are seen to have occurred in the STH group. Moreover, the distributions tend to become largely equal by the end of the 14-day period. It is conceivable that virtually the same per cent composition of the granulation tissue might have been obtained in all groups if the experiment had been continued.

In Plate I (between pp. 88 and 89) colour reproductions of micrographs representing the different groups after five and 14 days are presented. They show the circumstances stated above as they are revealed to the examiner.

7.6 Comments

In most studies concerning granulation tissue such tissue is observed to contain an ample number of leukocytes and giant cells, which confuse the microscopic picture. The profuse occurrence of such cells is a sign of inflammation and foreign-body irritation, which has sometimes been used to provoke formation of granulation tissue (Selye 1953, Stebbins and Stoerk 1955, Saikku 1956, Viljanto and Kulonen 1962). However, the difficultly controllable inflammation process often causes great disturbance of the repair (Dupont 1959), thus impeding the study of the effects exerted by extraneous factors, such as hormones. In the present work, formation of granulation tissue around the tendon was achieved without these annoying factors. The advantage of this is that no marked secondary inflammation phenomenon can obscure such hormonal effects as there may be.

It is evident already from clinical experience in itself that the granulation tissue becomes resistant against infection, i.e., against bacterial enzymes and toxins, at a certain stage. Likewise, its response to hormones obviously soon decreases as the tissue ages (Whipple 1940). According to the first law governing the general mechanism of action of hormones, hormones affect the intensity and rate of the granulation process (Williams 1955).

Table 2. Changes in relative quantity of fibrocytes, ground substance and collagen from 5 to 14 days.

Group	Fibrocytes			Ground substance			Collagen		
	5 days	14 days	Change, per cent	5 days	14 days	Change, per cent	5 days	14 days	Change, per cent
C	162	156	− 0.6	365	238	− 12.7***	235	372	+ 13.5***
STH	170	157	− 1.3	258	214	− 4.4***	336	428	+ 9.2***
TTH	233	160	− 7.3**	340	251	− 8.9***	182	373	+ 19.1***
STH + TTH	193	153	− 4.0***	461	276	− 18.5***	87	351	+ 35.1***

*** = Statistically highly significant, ** = significant, * = almost significant difference

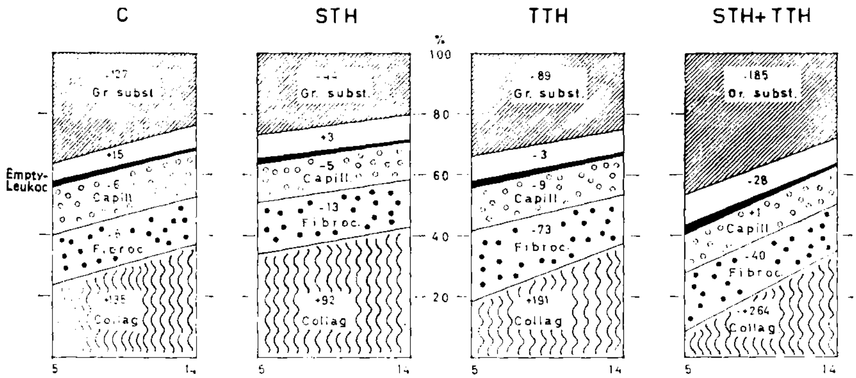


Fig. 39. Graphical presentation of the changes the relative amounts of different tissue components have undergone with progressing maturation of the granulation tissue. The most powerful change has occurred in the STH + TTH group. — The slope of the graphs reflecting the change is consistent with the corresponding tension test results; cf. particularly Fig. 21.

When the process is completed, hormones have no longer any influence on it.

In the tissue-analytic studies that were carried out, the differences between the hormonal treatment groups could be seen to have levelled out in the test series at fourteen days, which is consistent with the foregoing. The relative composition of the tissue in the different hormonal treatment groups then begins to resemble that of the controls. Of course, the regeneration taking place continuously in the organism is also subject to hormonal effect. This is thought to account for the fact that in various endocrinopathies despite the presence of a distinct clinical picture no pathological findings can be elicited on histological examination of the connective tissue. A stupendous disturbance is clearly needed for such manifestation to occur.

In the series of specimens taken at five days the relatively greatest amount of collagen was found in the STH group. Correspondingly, the quantity of ground substance was lowest in the same group. The action of somatotropin to cause increased formation of collagen has also been noted by Scow (1951) and Banfield (1958). The increased proliferation of fibroblasts reported by Taubenhaus and Amromin (1950) was not observed on the other hand. The increased collagen quantity observed here corroborates the opinion presented by Silberberg et. al. (1964) that somatotropin accelerates the development of the collagen fibres. The appearance of collagen in the wound is a sign of maturation of the granulation tissue (JACKSON 1958). In his histoquantitative studies, SAIKKU (1956) observed that somatotropin caused maturation of granulation tissue. In the present work the structure of the tissue displayed the least change in the period from five to 14 days just in the STH group, which speaks in favour of early maturation in these instances.

The inference can thus be drawn that somatotropin promotes the maturation of the granulation tissue and the collagen formation. In other words, *somatotropin accelerates and stimulates the second, or collagen, phase in the formation of granulation tissue.*

The action of thyrotropin is to increase the fibrocyte and mucopolysaccharide quantity and to reduce that of collagen. In the thyrotropin group the greatest number of fibrocytes was found in the series referring to five days, with statistically highly significant differences in comparison to the C and STH groups. In the literature, thyrotropin is stated to stimulate the mucopolysaccharide formation of connective tissue (ASBOE-HANSEN 1959). Increased amount of connective tissue cells could also be observed by WEGELIUS and JOKINEN (1962). GYLLING (1964) achieved with thyrotropin an increase in the relative ground substance quantity. This circumstance becomes visible in the 14-day series, where the relative ground substance quantity is clearly greater in the TTH than in the STH group, with a statistically highly significant difference. However, the conclusion is not thought to be appropriate that these agents would be antagonistic to each other. It appears likely that *thyrotropin stimulates the first, or productive phase of the formation of granulation tissue.*

Since it was reasoned that thyrotropin stimulates the productive and somatotropin the collagen phase in the formation of granulation tissue, the result of their simultaneous administration should be a great amount of granulation tissue. It is seen from the preceding and following chapter that this is indeed true.

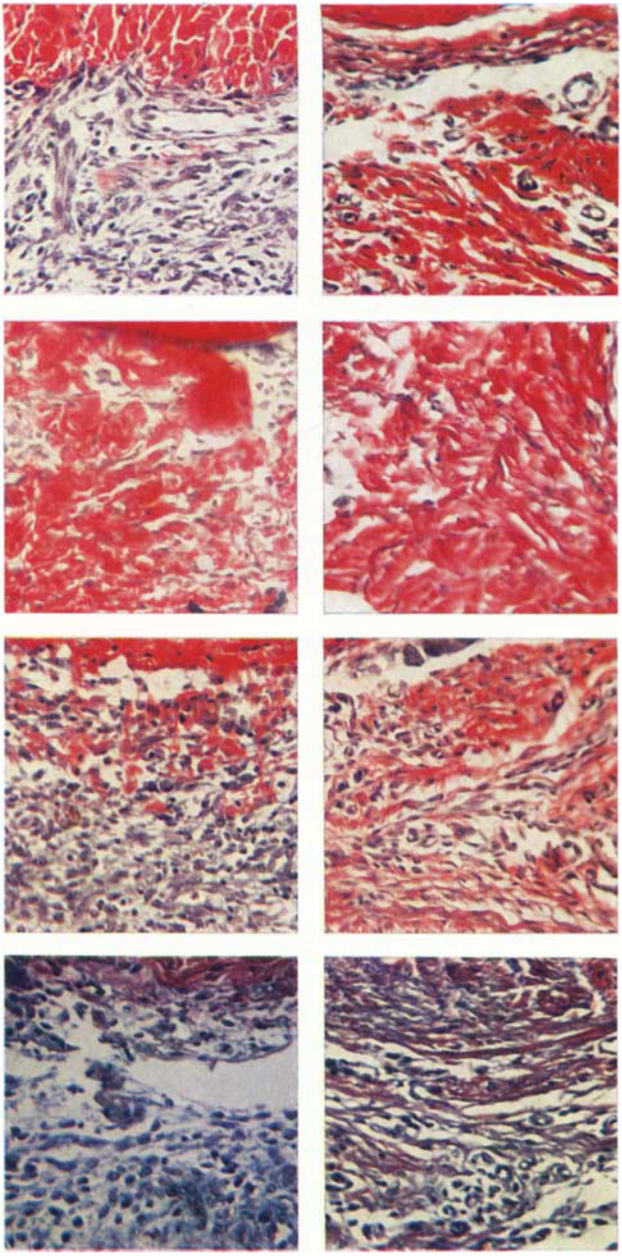
PLATE I (opposite).

Colour reproductions of micrographs from details adjacent to the tendon surface in the preparations (magnification $\times 400$). The tendon lies at the top of the micrographs. – When stained by Pentachrome II, collagen acquires an intense red colour and ground substance appears blue.

After five days, red collagen is already seen in ample amount in the somatotropin group. The group with synergistic action of somatotropin and thyrotropin still displays very little collagen at this stage, while the quantity of blue ground substance is high.

After 14 days, a high quantity of collagen is seen in all preparations, indicating maturation of the granulation tissue.

5 days | 14 days



C — C

STH — STH

TTH — TTH

STH + TTH — STH + TTH

7.7 Synopsis of the histological studies

1. In the test series after 5 days the following observations were made:

a) *Fibrocytes occurred in relatively greatest amount in the thyrotropin group, with statistically highly significant or significant differences from all other experimental groups. The quantity of fibrocytes in the somatotropin plus thyrotropin group was greater than in the control group at a statistically significant level.*

b) *Ground substance occurred most in the somatotropin plus thyrotropin group and it gradually decreased in amount in the control, thyrotropin and somatotropin groups. The quantity noted in the group with combined treatment differed from those in the control and somatotropin groups at a statistically highly significant level and from that in the thyrotropin group at a significant level. In the somatotropin group it was highly significantly less than in the controls.*

c) *The relative quantity of collagen was highest in the somatotropin group, which was followed by the control, thyrotropin and somatotropin plus thyrotropin groups, in this order. All groups displayed statistically highly significant differences from each other.*

d) The administration of hormones had no noteworthy effect on the quantities of capillaries, leukocytes and empty spaces.

2. In the test series after 14 days the following observations were made:

a) *In the relative amount of fibrocytes no statistically significant differences between the hormonal treatment groups could be noted, nor between them and the controls.*

b) *The occurence of ground substance was still highest in the somatotropin plus thyrotropin group, followed by the thyrotropin, control and somatotropin groups, but the differences between groups had largely levelled out. The control group still differed at a statistically significant level from the combined hormonal treatment and somatotropin groups.*

c) *The relative quantity of collagen was again highest in the somatotropin group, with statistically significant differences from the controls and other groups. The differences between the other groups had become equalized.*

d) No effect of hormonal medication on the relative quantities of capillaries, leukocytes and empty space was noted.

3. Comparison of the results obtained in the test series after 5 days and after 14 days revealed that *the relative quantity of collagen had increased in all groups, most of all in the somatotropin plus thyrotropin group and least in the somatotropin group. The relative amount of fibrocytes decreased during the period in question in all groups, most markedly in the thyrotropin group. The ground substance quantity showed a similar decrease, which was strongest in the group with combined treatment and least in the somatotropin group.*

8. COLLATION OF FINDINGS

8.1 Comparison of the results obtained in tension tests, planimetric studies and histoquantitative studies

The results yielded by the different methods of investigation can be approximately related with each other on the basis of Hooke's law, which is expressed by the formula

$$F = E \cdot A \cdot \frac{\lambda}{l}$$

The formula states that the force F producing a given elongation λ is proportional to this elongation and to the cross section area A of the specimen, and inversely proportional to the specimen's length l. The coefficient of proportionality E (the modulus of elasticity) is a constant dependent on the structure of the material and it is higher accordingly as the substance is more compact and firm. The strength of granulation tissue, again, depends on its relative collagen quantity (WHIPPLE 1940).

The relationships of elongation and force in the test series carried out after 5 and 14 days may be mutually compared by examining the forces consistent with a given elongation. The correspondence can only be approximate because the departure from linearity caused by the intermediate yielding was disregarded. It is also to be noted that, owing to destruction of the specimens in the tension tests, the planimetric data have been derived from a different set of specimens. In all groups the cross section area of the adhesions could be seen to diminish from 5 to 14 days, most of all in the STH + TTH group.

In the control group (C) the force consistent with a given elongation ($\lambda = 50$ units $= 8.33$ mm) was the same in the tests after 5 days and after 14 days. Since the cross section area had decreased, an increase in the relative amount of collagen must have taken place in the intervening time or the resistance against deformation (the product EA) would have gone down. This was indeed corroborated by the tissue analysis studies.

In the STH group, the force for one and the same elongation diminished slightly during the period from 5 to 14 days. This indicates that there has been a reduction of the EA product, which means that the relative quantity of the collagen did not increase very much. It is seen that according to the tissue analysis the percentage of collagen increased least of all in this particular group.

In the TTH group a distinct increase of the force from 5 to 14 days was noted. Since here, too, the cross section area was reduced, the relative collagen quantity must necessarily have increased quite considerably. This conforms to the finding on tissue analysis that the collagen percentage was doubled in this group.

The tensile strength in the STH + TTH group was lowest among all values at 5 days, but it increased to be the highest value at 14 days. This unexpected change took place in conjunction with a decrease in cross section area of the adhesions exceeding those in all other groups. These facts indicate a powerful increase of the collagen percentage. In fact, the tissue analysis showed that the relative amount of collagen has increased by a factor of 4 in this group.

The observation can thus be made that the results obtained by the different methods employed in this work are mutually well consistent in each individual point.

8.2 Elastic properties and composition of the granulation tissue

On the theory that the structure of a tissue, in the first place its content of given components, together with the cross section area determines its elastic constants according to some simple relationship, an attempt has been made to relate the apparent modulus of elasticity of the adhesion tissue to the relative collagen content and absolute cross section area in the different groups considered in this study. If such a relationship holds true with sufficient accuracy, one of the three quantities can be determined by calculation if the other two are known.

The formula involved is that representing Hooke's law, which we may write

$$E = \frac{F \cdot l}{A \cdot \lambda}$$

Table 3. Forces consistent with a given elongation ($\lambda = 50$ units, crorresponding to 8.33 mm elongation) in the different test series derived from the graphs in Fig. 23 (p. 52), for comparison of the results.

Group	Relative units		Absolute, g	
	5 days	14 days	5 days	14 days
C	165	165	1270	1270
STH	200	190	1540	1463
TTH	150	180	1155	1386
STH + TTH	125	220	962	1694

If the values in Table 3 previously derived from the graphs constructed on the basis of the mean relationship between the tensile force F and the elongation λ (cf. p. 52) are substituted, we find the figures stated in the tabulation below. It should be noted that these values of E are not consistent with any physical dimension in common use.

Group		E	Relative collagen quantity
C	5 days	239	0.235
STH	5 days	392	0.336
TTH	5 days	192	0.182
STH + TTH	5 days	89	0.087
C	14 days	306	0.372
STH	14 days	413	0.428
TTH	14 days	333	0.373
STH + TTH	14 days	202	0.351

Despite some deviations, *the two sets of values are obviously consistent in expected manner.* However, in view of the approximate character of all values involved in this consideration it is not thought to be appropriate to carry it to the otherwise logical conclusion of presenting positive formulae by which e.g. the strength properties of tissue could be calculated from measurements performed on its histological preparation, or the cross section area of the granulations from the strength and the tissue analysis data.

8.3 Absolute quantity of the tissue components

Since identical subjects were concerned in the planimetric studies and in the tissue analysis reported in the foregoing, these data can be considered in combination for each individual preparation and not merely on the mean value level.

When the per cent contribution of each tissue component to the entire cross section and the cross section area are known, the absolute area covered by each component can be calculated. This has been done for each individual preparation with respect to three tissue components, which were considered most important to be studied in this manner, namely, fibrocytes, ground substance and collagen. The means of the results from this computation with their standard deviations and variances have been presented in Tables 4—6 and in Fig. 40.

Table 4. Comparison of the absolute fibrocyte quantities in the granulation tissue, revealing statistically highly significant changes under the effect of hormones.

5 days	Var.	S.D.	Mean	Group		STH ↓	TTH ↓	STH +TTH ↓ , Difference compared to					14 days
	0.31	19	**68**	**C**	***	*		***	**C**	**50**	16	0.32	
	0.42	22	**52**	**STH**	**	*	**	***	**STH**	**42**	14	0.33	
	0.52	58	**111**	**TTH**	***	***	***	***	**TTH**	**53**	18	0.34	
	0.28	44	**156**	**STH +TTH**	***	***	***	***	**STH +TTH**	**95**	45	0.47	
	Difference, compared to				↑ C	↑ STH	↑ TTH		Group	Mean	S.D.	Var.	

Squares on diagonal between both halves of the table: Difference 5 days – 14 days

Unit: mg of paper cut-out weight

*** Statistically highly significant
** Statistically significant
* Statistically almost significant

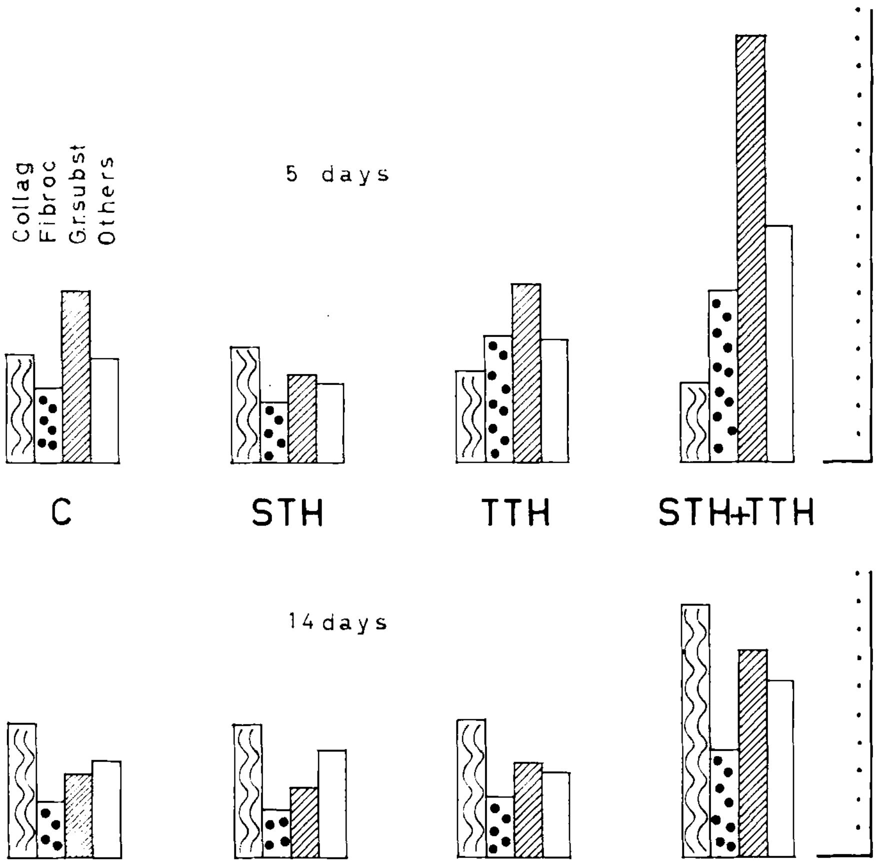


Fig. 40. Comparison of the absolute quantities of different tissue components in the granulation tissue after 5 and 14 days.

Statistical analysis of the results stated in the tables reveals highly significant differences between the values relating to five days and 14 days in all but the STH group and with respect to all tissue components considered here. In the STH group only an almost significant difference between five and 14 days is seen in all three quantities. This constitutes further proof in support of the action of somatotropin causing rapid maturation of the granulation tissue, as does also the small amount of cells and ground substance encountered in the preparations of the STH group. The collagen quantity is high on the other hand, which implies that *somatotropin obviously promoted the process of the collagen phase.*

Table 5. Comparison of the absolute ground substance quantities in the granulation tissue. Statistically highly significant changes are observed.[1])

	Var.	S.D.	Mean	Group		STH ↓	TTH ↓	STH +TTH ↓	, Difference compared to				
5 days	0.26	40	151	C	***	*		***	C	76	24	0.32	14 days
	0.35	27	77	STH	***	*	**	***	STH	62	28	0.45	
	0.34	54	158	TTH		***	***	***	TTH	83	28	0.34	
	0.25	95	377	STH +TTH	***	***	***	***	STH +TTH	184	18	0.42	
	Difference, compared to				↑ C	↑ STH	↑ TTH		Group	Mean	S.D.	Var.	

Squares on diagonal between both halves of the table: Difference 5 days – 14 days

Unit: mg of paper cut-out weight

*** Statistically highly significant
** Statistically significant
* Statistically almost significant

Table 6. Comparison of the absolute collagen quantities in the granulation tissue. Statistically highly significant changes are observed.[1])

	Var.	S.D.	Mean	Group		STH ↓	TTH ↓	STH +TTH ↓	, Difference compared to				
5 days	0.25	24	97	C	***			***	C	120	32	0.38	14 days
	0.33	31	101	STH		*		***	STH	117	36	0.31	
	0.25	20	83	TTH	**	**	***	***	TTH	121	30	0.25	
	0.34	24	71	STH +TTH	***	***	*	***	STH +TTH	223	65	0.29	
	Difference, compared to				↑ C	↑ STH	↑ TTH		Group	Mean	S.D.	Var.	

Squares on diagonal between both halves of the table: Difference 5 days – 14 days

Unit: mg of paper cut-out weight

*** Statistically highly significant
** Statistically significant
* Statistically almost significant

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[1]) Closer study of the tables 4 – 6 opens many new, interesting perspectives. No comments are presented on such aspects since they go beyond the problems outlined for the present study.

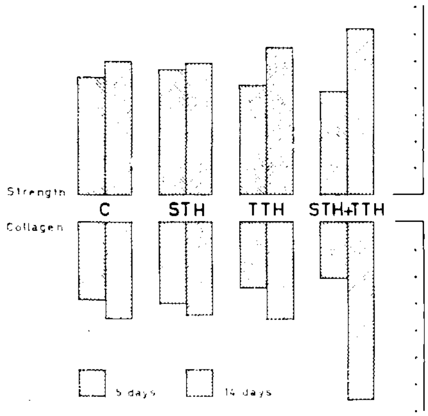

Fig. 41. Ultimate tensile strengths recorded in the different test series compared to the absolute collagen quantities. Both component diagrams are seen to be very close mirror images of each other.

In the thyrotropin group fibrocytes are seen to occur in greater number at five days than in the C and STH groups, in a degree which amounts to a statistically highly significant difference. Also ground substance is encountered in ample amount. As no such differences of the TTH group from the other groups exist at 14 days, the inference can be drawn that *the target of the action of thyrotropin is the first, or productive, phase of granulation formation.*

The STH + TTH group displays statistically highly significant differences in all three tissue component quantities in comparison with all other groups. When somatotropin and thyrotropin are administered simultaneously, the fibrocyte proliferation and formation of ground substance are enhanced at first; at a later stage collagen is produced in ample quantity.

Collagen constitutes the framework holding the granulation tissue together. It is worth while to compare the established absolute collagen quantities to the corresponding ultimate tensile strengths. When this is done for the mean values of the different subgroups, plotting the two quantities against each other, a diagram suggesting *fairly good linear correlation* is obtained. This is brought home convincingly by the presentation in Fig. 41 in that the values of one quantity very faithfully mirror those of the other.

9. DISCUSSION

In the comments on the results stated in the preceding chapters the problem of tendon adhesions was discussed on the basis of the line of research involved in each particular chapter. Due note was taken of the fact that tension tests, planimetric studies and studies by means of tissue analysis complement each other and that their respective results can be substituted as numerical quantities in an equation binding them together. This provides a means by which a comprehensive idea of the phenomena in question is easier to obtain.

Before embarking on a conclusive discussion of the tendon adhesions and their behaviour in the conditions of the present experiments, the general effects exerted by somatotropin and thyrotropin on granulation tissue shall be considered on the basis of the studies carried out in the present work. The fact should be kept in mind that a biological process of fundamental nature, as for instance the formation of granulation tissue, is invariably acted out to its ultimate conclusion also without assistance from exogenous hormones; administered hormones are thus only able to influence the intensity of the various events and the rate at which they take place (WILLIAMS 1955).

Somatotropin was found to accelerate the maturation of the granulations by stimulating the collagen formation. The following circumstances noted in the test series after five days of the various treatments and in that after 14 days are in support of this inference.

– Establishment of the highest ultimate tensile strength in the five-day test series with somatotropin is indicative of rapid maturation.

– The tensile strength in the somatotropin group showed the least change during the period from 5 to 14 days.

– The firm tissue with high collagen content presented the least ultimate elongation in both series.

– The granulations matured within a short time and ceased to grow after that, with the consequence that their cross section was smallest in either series.

— Mature granulation tissue no longer increases in any noteworthy degree; consistently, the change in cross section area from 5 to 14 days was least with somatotropin.

— The collagen percentage was highest in both test series.

— Immature ground substance occurred least in both series.

— The composition of the mature granulation tissue underwent the least change between the times of 5 and 14 days.

— The absolute quantity of collagen increased least of all during the period from 5 to 14 days.

The present studies thus corroborate and complement the opinion presented before by Scow (1951), Spain and Molomut (1953), Saikku (1956), Banfield (1958) and Silberberg et. al. (1954), that somatotropin accelerates the collagen formation.

Thyrotropin was found to stimulate the productive phase of connective tissue formation, accelerating the proliferation of fibrocytes and the formation of ground substance. The following circumstances relating to this group in the tests after five days and after 14 days bear evidence of this.

— The low ultimate tensile strength after five days indicates that the productive phase is still in progress and there has been little collagen formation.

— Immaturity of the tissue at five days seems to be borne out by the powerful increase in ultimate tensile strength between 5 and 14 days.

— A high value of the ultimate elongation was recorded in the five-day series.

— The adhesions were large in cross section area, evidencing lively formation of connective tissue.

— The percentage of collagen was still low at five days.

— The quantity of ground substance was high.

— The highest number of cells at five days was recorded in this group.

— Suggestive of immaturity of the tissue are the great changes it underwent between 5 and 14 days.

— The absolute number of cells was high.

— The absolute quantity of ground substance, too, was second highest among the different groups.

It should be noted that the organism possesses an efficient regulating mechanism, which counteracts the influence of thyrotropin (Brunish 1960).

The contention that thyrotropin promotes the production of connective tissue is also supported by IVERSEN and ASBOE-HANSEN's (1952) and ASBOE-HANSEN's (1959) observation of increase of the mucopolysaccharides. Another indication to this effect is the formation of exophthalmus (BOAS and SOFFER 1950, DOBYNS and STEELMAN 1953).

On simultaneous administration of *somatotropin and thyrotropin,* stimulation of the connective tissue production was observed at first, and then of collagen formation. The following circumstances noted in the test series after five days and after 14 days, for instance, speak in favour of initially lively cell division and formation of ground substance.

— The tensile strength of the tissue was lowest of those recorded in the five-day series.

— The change in tensile strength during the period between 5 and 14 days was greatest in this group.

— The yielding of the loose tissue had the highest value in the five-day series.

— The ultimate elongation was largest among those referring to five days.

— Much granulation tissue was formed, and the largest adhesion cross sections were recorded.

— The lowest collagen percentage at five days occurred in this group.

— The lowest absolute collagen quantity at five days occurred in this group.

— Cells were noted in ample number.

— The quantity of ground substance was highest in the five-day as well as the 14-day series.

The following indications of subsequent invigoration of the collagen formation were obtained.

— Highest tensile strength in the 14-day series.

— Highest increase of tensile strength from 5 to 14 days.

— The yielding was no longer highest in the test after 14 days.

— The compacted tissue was not elongated most of all in the 14-day series either.

— The highest absolute collagen quantity in the 14-day series was recorded in the somatotropin plus thyrotropin group.

It is concluded that much granulation tissue has time to be formed and the strongest adhesions are produced after collagen has appeared in the picture. In connection with fractures of the long bones this phenomenon has caused massive, hypertrophic callus formation (KOSKINEN 1959).

One has to exercise certain caution in any attempt to apply the experimental results in actual clinical practice. The fact has already been pointed out that the tendon consists of highly differentiated tissue, which therefore has low regenerative ability. As a matter of fact the repair of a tendon takes place from the periphery and indeed by means of the adhesion formation (WEHNER 1923, HUECK 1923, NÄRVI 1926, SKOOG et. al. 1954, ASHLEY et. al. 1960, POTENZA 1962). What has been said above is therefore also applicable in cases in which it is desired to restore the continuity of a tendon. In actual truth the formation of adhesions is then desirable, because it alone is able to knit the severed ends together once more. Any attempt at reconstruction of a severed tendon involving endeavours to *block* the formation of adhesions, e.g. by means of interpositions (WECKESSER et al. 1949, MCKEE 1945, STINCHFIELD 1950, LEVEEN 1949), is foredoomed to failure. The real aim should be to *control* the formation of adhesions.

In tendorrhaphy the desirable events are: rapid consolidation, early collagen formation, and a small scar. In this light the use of somatotropin in connection with tendorrhaphies would seem to be indicated. Cortisone has been found to prevent the formation of adhesions, resulting in separation of the tendon fragments (CARSTAM 1953). If the aim is to attach the tendon firmly to the bone or to perform tenodesis, a strong, massive scar is desirable. Administration of somatotropin in combination with thyrotropin would then seem appropriate. In tenolysis, again, early formation of a scar is not wanted. In such instances thyrotropin alone can be administered. The writer's clinical experiences, which are small in extent so far, are encouraging (Fig. 42).

In general surgery, too, e.g. in various hernias, a massive and firm scar is desirable. This is an instance in which combined hormonal treatment may be tried out. The said hormones are also used in thorax surgery (UOTILA, PERÄSALO and VAPAAVUORI 1955) and expressly in tuberculosis (CARSTENSEN, PAULSEN and RUDBERG-ROOS 1954).

In soft tissue surgery the administration of hormones has the advantage that they need be given during a short period only. This implies that immunity has not time to develop, and no general effects can become established. It should be stressed, however, that atraumatic technique (BRUNELL 1921) and aseptics are absolutely primary in surgery on the limbs and cannot be replaced with any hormonal expedients.

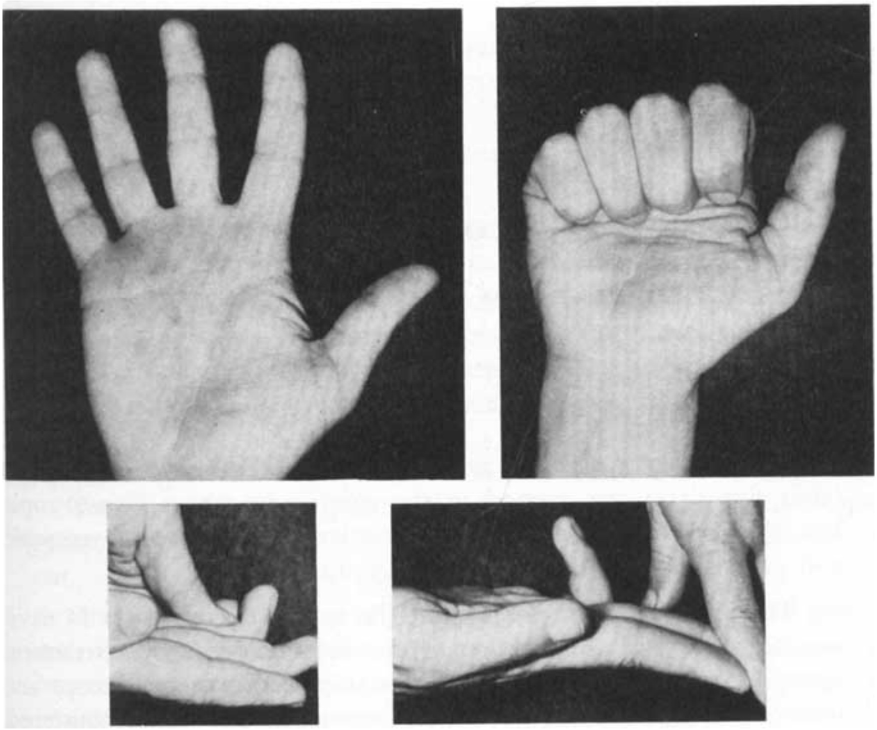

Fig. 42. Patient B.H., 37 years, male. Both flexor tendons of the fourth digit were cut through in the distal part of the palm. After primary tendorrhaphy, the patient was given 20 U.S.P. units somatotropin (»Somacton», Ferring Ab., Malmö, Sweden) every second day during two weeks. Absolutely complete restoration of the function of both tendons was achieved.

In various endocrinopathies, such as acromegaly, excessive growth and fibrosis are encountered as a consequence of overabundant secretion of the hormones in question. The regeneration of connective tissue is stimulated, and the skin is thick and high in collagen content (Asboe-Hansen 1954). The writer is somewhat inclined to consider also pathological states such as cheloid tendency and Dupuytren's contraction to be secondary manifestations of hormonal disturbance.

For the behaviour of granulation tissue under the effect of hormones a certain numerical expression could be found in the studies reported here. Future work will no doubt show the extent in which it can be usefully employed in clinical research. In addition to surgical trauma, also the effects of endocrinectomies, adrenal hormones, vitamins and other factors may be illuminated by the procedures applied in this work.

10. CONCLUSIONS

In the studies that have been carried out, thyrotropin, somatotropin and the combination of both were found to have a distinct effect on the adhesions formed around a traumatized tendon of the rat. The following can be stated in answer of the questions outlined earlier in this work.

1. (a) The ultimate tensile strength at the end of the productive phase (after five days) was highest in the animals to whom somatotropin had been given, followed in declining order by the control, thyrotropin and somatotropin plus thyrotropin groups.

   (b) The ultimate tensile strength in the collagen phase (after 14 days) was highest in the combined somatotropin plus thyrotropin treatment group. It was clearly less in the thyrotropin and control groups and least of all with the animals to whom somatotropin had been administered.

   (c) The increase in ultimate tensile strength during the period from 5 to 14 days was greatest in the group with combined somatotropin and thyrotropin treatment and least in the somatotropin group.

   (d) The hormonal treatments could not be observed to exert any statistically significant effect on the elongation.

2. (a) The cross section area of the adhesions after five days was largest in the somatotropin plus thyrotropin group, followed in declining order by the thyrotropin, control and somatotropin groups.

   (b) The same order of the groups as above was noted in respect of cross section area of the adhesions after 14 days.

   (c) The cross section area of the adhesions decreased during the time from 5 to 14 days in all groups. The change was least in magnitude with the animals under somatotropin treatment.

3. (a) Histological examination after five days revealed the following facts.

— The relative collagen quantity was highest in the somatotropin group, followed by the control, thyrotropin and somatotropin plus thyrotropin groups.

— Fibrocytes occurred in greatest abundance in the group of animals under thyrotropin treatment.

— Ground substance was found in relatively highest quantity in the somatotropin plus thyrotropin group; this quantity decreased gradually according to the succession: controls, thyrotropin group, somatotropin group.

— No differences between groups were noted in respect of any other tissue components present in the adhesions.

(b) Histological examination after 14 days revealed the following facts.

— The relative collagen quantity was again highest in the somatotropin group, but the differences between the other three groups had levelled out.

— The greatest abundance of ground substance was noted in the group with combined somatotropin and thyrotropin treatment. The differences between groups were becoming less also in respect of the relative ground substance quantity.

— No differences between the different groups were observed in regard of any other tissue components, including the relative fibrocyte quantity.

(c) The following changes took place in the histological structure of the tissue during the period from 5 to 14 days.

— The relative collagen quantity increased in all groups, most of all in that with combined somatotropin and thyrotropin treatment and least of all in the group with exclusive somatotropin administration.

— The number of fibrocytes decreased in all groups, most strongly in the thyrotropin group.

— The quantity of ground substance, too, decreased in all groups. The change was again greatest in the group with combined treatment and least in the somatotropin group.

4. The differences in tensile strength established between the different groups and the change of the tensile strength between 5 and 14 days were found to correlate with the changes in cross section area and structure of the tissue. Owing to the relationship between the results

produced by the different methods, one of the three quantities could be mathematically determined when the values of the other two were known.

5. The granulation coming into existence around an injured tendon is subject to the same laws as the healing of a wound in general. Hormones were concluded to have the following influence on the formation of granulation tissue.

(a) Thyrotropin stimulated the productive phase of granulation formation.

(b) Somatotropin promoted the collagen formation and caused rapid maturation of the granulation tissue.

(c) Simultaneous administration of thyrotropin and somatotropin at first accelerated the production of granulations; at a later time collagen was formed in profusion.

11. SUMMARY

The adhesions produced around a traumatized tendon and the effects exerted on them by the hypophyseal hormones somatotropin and thyrotropin have been studied in experiments on the rat's Achilles tendon. Both hind legs of 355 experimental animals were subjected to operation involving trauma to the tendon. One series of studies was made five days after the traumatizing operation and another after 14 days. Both series include four groups, namely, the control, somatotropin, thyrotropin and combined somatotropin plus thyrotropin groups.

Three different methods of investigation were employed, the results of which can be brought into mutual relationship, thus enabling them to be checked to a certain extent.

1. ***Tension tests.*** The tensile force acting on the tendon and on its adhesions was recorded as a function of the elongation in a specially designed tension testing apparatus. The tendon was invariably first detached from the bone; a force four times as high was required to overcome the soft tissue adhesions. This has been ascribed to the fact that the tendon adjoins on bone by a small part of its area only, and to the small ultimate elongation of those adhesions.

At the end of the productive phase (after five days) the highest ultimate tensile strength was noted in the somatotropin group, which was followed by the control and thyrotropin groups, while the least force was required in the somatotropin plus thyrotropin group.

In the collagen phase (after 14 days), paradoxically, the highest tensile strength was obtained in the group with combined somatotropin and thyrotropin treatment. The increase was considerable also in the thyrotropin group, which left the control group behind. The increase was least in the somatotropin group, for which the lowest value was recorded at 14 days.

2. The ***cross section area*** of the adhesions was studied by a planimetric method involving the weighing of paper cut-outs.

In contrast to the ultimate tensile strength sequence at five days, the somatotropin group presented the smallest cross section of the adhesions. The area was greater in the controls and in the thyrotropin group and the highest value was found in the somatotropin plus thyrotropin group.

In the course of maturation of the granulations, during the period from 5 to 14 days, the cross section area decreased in all groups, least of all in the somatotropin group. The mutual order of the groups in respect of cross section area did not change and was therefore the same at 14 days as that in the tension tests at 14 days.

3. ***Tissue analysis*** was employed in order to elucidate the structure of the granulation tissue, which is another factor determining the magnitude of the tensile strength, together with the cross section area. It involved the line sampling procedure and use of a Leitz integrating micrometer eye-piece. The components undergoing the most profound changes under the effect of the hormones in question were found to be collagen, fibrocytes and ground substance. The changes occurring in the quantities of other tissue components were negligible.

In the five-day series the somatotropin group presented the relatively highest quantity of collagen, surpassing the controls and the thyrotropin group. In the group of animals under combined hormonal treatment the tissue still mainly consisted of gelatinous ground substance; accordingly it failed under the lowest force in the tension test. The order of the different test groups by relative abundance of ground substance was opposite to that based on the collagen quantity. The greatest number of fibrocytes was encountered in the thyrotropin group.

According to the findings at 14 days, the relative collagen quantity had markedly increased in the somatotropin plus thyrotropin group, while the quantity of ground substance had decreased. The differences between groups had largely levelled out.

4. From the results concerning percentage of collagen and cross section area of the adhesions the absolute collagen quantity in the granulations could be calculated. The magnitudes of the ultimate force needed to detach the adhesions in the different groups at five days as well as at 14 days were seen to be closely reflected by the absolute collagen quantity in each instance. An explanation has thus been furnished for the observed changes in ultimate tensile strength during the period from 5 to 14 days and the seeming paradox is accounted for.

The material characteristic (apparent modulus of elasticity) occurring as coefficient in the expression which represents the force as a quantity proportional to the cross section area and elongation and inversely proportional to the length of the specimen under tension proved to be consistent with the collagen quantity. A system has thus been established in which a given numericity can be assigned to the granulation tissue formed around an injured tendon.

5. The results of the present studies are generally applicable to the behaviour of granulations under hormonal effect. The following ultimate inferences seem justified in this respect.

- Somatotropin causes maturation of the granulation tissue and accelerates the formation of collagen.
- Thyrotropin stimulates the productive phase in the formation of new connective tissue.
- Simultaneous administration of somatotropin and thyrotropin stimulates, at first, the production of granulation tissue and thereafter accelerates the collagen formation.

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CORRECTIONS

Page 1: for DEPARMENT place DEPARTMENT
Page 5: for 2.2 place 2.3
Page 6: for 8,3 place 8.3
Page 10: for BARBER place BARBERIA
Page 13: for WILLIAMSON and DUSCHLBAUER place WILLIAMSSON and GUSCHLBAUER
Page 15: for BIRD place BIRD and MACKAY
Page 16: for BODVAL place BODVALL
for SUNDBERG place SANDBERG
Page 18: for SKOOG place SKOOG and PERSSON
Page 19: for SKOOG place SKOOG and PERSSON
Page 20: for intances place instances
Page 24: for TENGROTEN place TENGROTH
for FRIEGOOD place FRIEDGOOD
Page 26: for TRAUBENHAUS place TAUBENHAUS
Page 30: for 0.001 (1 %) place 0.001 (0.1 %)
Page 59: for Goup place Group
Page 86: for DUPONT place DUMONT
Page 98: for SILBERBERG et.al. (1954) place SILBERBERG et al. (1964)
Page 100: for SKOOG et al. place SKOOG et PERSSON
for LE VEEN place LE VEEN et BARBERIA
for BRUNELL place BUNNELL
Page 110: for Dupuytresis place Dupuytren's
Page 112: for ILVESSALO -- 1961 place ILVESSALO -- 1921
for YONG place YOUNG