1955

The Effect of Hormones on the Rate of Healing of Experimental Wounds

George J. Neumann

Loyola University Chicago

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THE EFFECT OF HORMONES ON
THE RATE OF HEALING OF
EXPERIMENTAL WOUNDS

by

George J. Neumann

A Thesis Submitted to the Faculty of the Graduate School of Loyola University in Partial Fulfillment of the Requirements for the Degree of
Master of Science

February
1955
George J. Neumann was born November 27, 1928, in Mineola, New York. He attended high school in South Glens Falls, New York. The high school diploma was received in June, 1946.

The author served with the United States Army for a period of three years (1946-1949) during which time he was assigned to the 10th Chemical Base Depot, Eighth Army.

In June, 1952, he received the bachelor of arts degree from Champlain College, State University of New York.

Graduate study at Loyola University was begun in September, 1952.
ACKNOWLEDGEMENT

The author wishes to express his sincere appreciation for the many helpful suggestions and the aid given by Dr. Martin B. Williamson in the development of the problem. He also wishes to express his gratitude to the members of the faculty of Loyola University who have aided and encouraged him during his period of attendance at the University.
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CHAPTER I

INTRODUCTION

Growth is generally defined as an increase in the mass of an organic system. According to this definition growth may occur in two ways: 1) by increasing the mass of a unit through the assimilation of materials from the environment, and 2) by the increase in the number of units. Thus, any process by which the mass, or number, of individual units is increased may be considered to be "growth". The healing of wounds may therefore be considered to be a growth process. However, since the main interest of this thesis lies in a specialized process, it might be desirable to limit the definition even further. The definition, as given, includes such processes as the absorption of water and of inorganic salts and simple sugars which may elicit an increase in the mass of the system being considered. For example, a seed may "grow" by the absorption of water even though the seed is incapable of germination. In order to exclude effects such as this from consideration, Weiss (1949) has defined growth as "the increase in that part of the molecular population of an organic system which is synthesized within that system."

According to this definition, growth and reproduction are essentially synonymous. The replacement of hair, skin, nails, etc. which have been lost through "wear and tear" is also essentially synonymous with growth; as is morphallaxis, epimorphosis, and the healing of wounds. If this is true, where
do the processes differ?

That the processes do differ is obvious when one considers the extent to which the various processes proceed and the time during the life of the individual in which the processes may occur. Maturation, for example, involves the growth of the entire individual, while epimorphosis may be said to be the growth, or regrowth, of a localized portion of an individual. Maturation may be differentiated from wound healing by the fact that maturation occurs only during a certain portion of the life of the individual, while the healing of wounds may occur during any period of the individual's life.

Furthermore, Weiss' definition does not limit the number of molecular mechanisms by which growth may proceed, nor does it specify any particular physiological environment, i.e. composition of blood, the presence or absence of hormones or small ions or molecules.

Thus, while the end result, i.e. synthesis of large molecules, may be the same, the processes leading to these end results may differ. One may tentatively assume, then, that those factors which influence one form of growth also influence the other forms in approximately the same manner. Species, sexual and tissue differences may be assumed to modify this generalization.

Factors Influencing Wound Healing

The relation of age to the rate of healing of wounds has been studied by Du Nouy (1919). In defining the rate of regeneration of human skin, he has developed the equation:

\[ S = S_0 e^{-k(t-t^2/2p)} \]

where \( S_0 \) is the area of the wound at the time of production of the wound; \( S \) is the area of the wound at time \( t \), \( e \) is the base of natural logarithms and \( k \) and
p are constants. The constant k is said to indicate the age of the patient. Thus the ratio $S/S_0$, which is a measure of the rate of healing, declines as k, or the age of the patient, increases. The same generalization may be applied to the growth of an individual to a mature organism.

Certain dietary factors have been shown to influence wound healing as well as growth. Clark (1919) reported that a high protein diet decreased the time required to close skin wounds in dogs. Other workers have confirmed this work (Harvey, et.al., 1930; Thompson, et. al., 1938; Localio, et. al., 1943, 1948; Williamson, et. al., 1951).

A number of workers have reported on the effects of various amino acids on the rate of healing of wounds. Morris and his coworkers (1945) have shown that lysine, tryptophane, and valine have no significant effect on wound healing. Furth and Scholl (1937), however, report that tryptophane markedly accelerated the healing of duodenal ulcers in dogs and that glutamic acid and glycine gave negative results, while histidine and alanine were of doubtful benefit. Histidine has been shown to have no effect on the rate of healing of experimental wounds (Williamson and McCarthy, 1952). The work of a number of investigators has shown that methionine increases the rate of healing (Localio, et. al., 1949; Williamson and Fromm, 1952, 1953; Perez-Tamayo and Ihmen, 1953). Williamson et. al., (1951) and Fromm (1952) have reported that no apparent relation between the amount of protein nitrogen fed or retained and the rate of healing was noted in their experiments, but that there did appear to be a definite correlation between the rate of healing and protein sulfur retention.

Since vitamins play a vital role in metabolism, it is not surprising to find that a number of them have been found to influence wound healing.
General avitaminosis appears to delay healing (Ishido, 1923; Lauber, 1934). Saitta (1929) has demonstrated that wounds in guinea pigs on a scorbutic diet do not heal as rapidly as those in guinea pigs receiving the same diet containing a vitamin C extract. Since Saitta's experiments other workers have shown that vitamin C plays an important role in muscle regeneration (Murray and Kodicek, 1949), in the differentiation of bone (Murray and Kodicek, 1949; Bourne, 1942), and in mammalian skin wounds (Bradfield and Kodicek, 1949). Biotin, pyridoxin and riboflavin have also been reported to promote wound healing (Bosse and Axelrod, 1948) while vitamin D appears to be actively inhibitory (Saitta, 1930; Holmes, 1942; Rose, 1944). The data of Findlay (1953) appears to indicate that vitamin $B_{12}$ accelerates the rate of healing in the early stages of wound healing but has no effect on the later stages. This accelerating effect was not noted in protein depleted rats.

One of the most important factors which delay wound healing is infection (Du Nouy, 1936; Arey, 1936; Needham, 1952). According to Needham (1952) defense and demolition of injured cells must be complete before healing can begin. Carrel (1924) and Kiser (1927) have reported a delay in healing even when an abscess is present in an area distal to the wound.

It has also been reported that temperature (Ebeling, 1922; Reider, 1924), radiant energy (Schwarz, 1933; Nathanson, 1944), antiseptics (Anderson, 1938), detergents (Anderson, 1938; Scheinberg, et al., 1948) and irritants (Anderson, 1938) appear to effect the rate of healing of wounds.

The effect of hormones on the healing of wounds will be discussed at greater length in chapter II.
Measurement of the Rate of Healing

It is important to consider the techniques by which one may measure the rate of healing. Since the different methods of measurement apparently measure different events which occur during the healing of the wound, the events of the healing process will be briefly discussed.

The healing process may be divided into two main phases; the regressive phase and the progressive phase. Considerable overlapping of the two phases may occur.

Although wound closure may be considered to be part of the regressive phase, it usually occurs very shortly after the production of the wound. Closure may be effected in two ways: 1) by apposition, where the edges of the wound are brought together by a stretching of the cells to bridge the wound gap, adhesion, and subsequent contraction of the cells to close the wound, 2) by scab formation in those wounds which are too large to be spanned by the simple stretching of the cells. In this case the wound is closed by the formation of a fibrin clot which dries, with filterable elements of the blood and tissue debris, to form a scab. The scab is then permeated by epithelial tissue and is eventually lost. (Arey, 1936; Needham, 1952).

Cells other than those destroyed by the mechanical production of the wound may also be damaged (Arey, 1936; Needham, 1952). This damage is usually due to either oxygen starvation or poisoning by foreign matter (Needham, 1952). Removal of the injured cells is accomplished partially by autolysis and partially by phagocytosis. Foreign organisms which invade the area are probably destroyed by antibodies and phagocytes. As mentioned previously, further healing cannot take place before defense and demolition have been completed.
(Needham, 1952). When the defense-demolition period has been completed, it may be said that the regressive phase has ended and the progressive phase begun. Usually the regressive phase of the healing process is short.

During the progressive phase, differentiation of lymphocytes, monocytes and fixed macrophages to fibroblasts is observed. The fibroblasts are in some manner related to the elaboration of collagen fibers. Arey (1936) has considered these relations in more detail.

With this brief summary of the events of wound healing in mind, it is obvious that one way in which the progression of healing may be followed is by histological observation. The greatest advantage of this method lies in the fact that one may observe which portion of the healing process is affected by the factors being observed. The method is probably the most widely used technique. There are objections to the method, however. Where the number of wounds being studied are large, the method becomes tedious. Furthermore, the method is not quantitative and allows for a great deal more subjective error than other techniques.

The measurement of the decrease of the area of the wound has been used by a number of investigators (Morris, et. al., 1945, Dunn, 1945, Baker and Whitaker, 1950). One modification of this technique involves the production of a circular wound about which is sutured support to minimize the passive stretching or shrinking of the wound. Sterile technique is necessary to minimize the danger of infections. Dressings are applied and changed at intervals for the same purpose. Area measurements are made at intervals and the reciprocal plotted against time to give a sigmoid curve of the type shown in Figure 1. The chief advantage of this technique is that the same animal may be
Fig. 1. A typical growth curve. The ordinate may be tensile strength, wound area, number of cells, etc.
used throughout the study. However, there are a number of disadvantages. The method obviously measures only the rate of closure of the wound, which is effected mainly by the migration of epithelial cells (Arey, 1936). Since the rate of migration of epithelial cells does not necessarily parallel the rate of formation of structural materials, no information concerning these materials may be obtained with this method. Furthermore, the wound does not heal circularly but tends to become elliptical and finally approximates an incision, thus making area measurements difficult. Another difficulty is the excessive handling of the animal. These considerations discourage the use of the technique.

As early as 1918 it was shown that a potential difference was exhibited between an injured area and normal skin (Melchior and Rahm, 1918). These potentials have been used to measure the rate of healing of human skin wounds (Barnes, 1945, 1946, 1951). The method is highly acceptable where practical. However, since the method requires placing the wound in a conducting medium, it would seem that the method would be impractical in measuring healing rates in experimental animals. If laboratory animals were used, one would have to restrain the animal in some fashion since movements may invalidate the results (Barnes, 1951). The restraint may, of itself, invalidate the results by inducing trauma, which is to be avoided if possible. Many extraneous factors seriously affect the wound potentials.

Because of the limitations of the methods discussed above, wound strength measurements were used to follow the rate of healing in this investigation. The apparatus which was used is a modification of that described by Charney, Williamson and Bernhart (1947). The apparatus will be described more
fully in a later section. The method measures the force required to pull the
wound apart. When the wound strength is plotted against time, a typical
growth curve (Figure 1) is obtained (Kobak et. al., 1947; Howes et. al., 1933).
The portion of this curve between points A and B approximates a straight line,
the slope of which is a measure of the rate of healing of the wound (Fromm,
1952). The strength of the wound depends on the amount of structural materials
in the wound and therefore measures directly the production of the structural
materials of the wound. This method combines validity with rapidity and
simplicity and requires little handling of the animal once the wound has been
produced. Subjective error is also reduced in this technique. The method is
not without its faults, however, some of which have been discussed by Taylor
and Zipperman (1950). A list of some of the variables which contribute to
deviations in the wound strength determinations in the wound strength determina-
tions are: inadvertent variations in the width of the section of the wound
taken for determination, variability in the method of fixing the tissue in the
apparatus, the variable tearing of the wound and fluctuations in the rate of
flow of mercury into the container. These variations, however, do not invali-
date qualitative conclusions except where differences in the wound strength are
quite subtle.
CHAPTER II

THE INFLUENCE OF HORMONES ON THE RATE OF HEALING

A large number of clinical observations and a great deal of laboratory investigation has established the importance of hormonal balance for normal growth. Since the clinical manifestations of hormonal imbalances are described in detail in a number of books on endocrinology and pathology, a description of these manifestations will not be attempted in this thesis. It will suffice to mention that, among other disorders hypofunction of the anterior pituitary in young individuals is accompanied by dwarfism, while hyperfunction leads to giantism or to acromegaly in individuals who have ceased growing before the onset of the disorder and that hypothyroidism may also lead to dwarfism.

Laboratory investigations have shown that hormones affect general metabolism and enzyme activity. Long et. al. (1940) and Anderson (1943) and Evans (1936) have shown that the adrenalectomized rat cannot readily utilize endogenous protein during a fast. Furthermore, in order that the adrenalectomized rat survive, sodium chloride must be supplied to the animal (Kendall, 1943). Ingle (1941) and Ingle, Li and Evans (1946) have shown that cortisone and ACTH are capable of inducing glycosuria and hyperglycemia in rats, which was accompanied by an increased nitrogen excretion.

Kochakian (1946) reported that estradiol injections increased kidney weights and renal arginase, although to a lesser extent than androgens. Sawyer and Everett (1946) found that serum cholinesterase levels paralleled estrogen
activity.

From the few facts presented, one can appreciate the importance of hormones in maintaining a normal metabolic pattern in the individual. One is led, therefore, to expect that hormones should exhibit an effect on the rate of healing and the normal progression of wound healing.

**Adrenal Hormones**

A number of reports have indicated that the administration of cortisone induces an inhibition of healing (Shapiro et al., 1951, Spain et al., 1950, Ragan et al., 1949, Taubenhaus and Amromin, 1949, 1950). Baker and Whitaker (1950) have reported that cortisone did not significantly retard wound closure. This does not necessarily constitute a contradiction since as mentioned previously, the rate of migration of epithelial cells does not necessarily parallel the rate of formation of granulation tissues. In their experiment, Baker and Whitaker also observed the wound histologically and report that cortisone had a marked effect on the granulation tissue. Taubenhaus and Amromin (1950) reported that cortisone had an inhibiting effect on fibroblasts and collagen formation. Pirani et al. (1951) observed the same type of situation in investigating the effect of deoxycorticosterone on the healing process. These workers report that the hormone induced the appearance of a great number of fibroblasts which was accompanied by a "slight to moderate" lag in the maturation of both cellular and intercellular elements. Taubenhaus, Taylor and Morton (1952) have made essentially the same observations. Thus, it may be said once again, that the processes of epithelization, fibroblast appearance, and collagen formation do not necessarily parallel each other.
Shapiro, Taylor and Taubenhaus (1951) have investigated the possibility of cortisone action being mediated through a nervous mechanism or through the development of ischemia. They have concluded that the action of cortisone is a direct one and is not due to ischemia or a nervous mechanism.

Ragan et al. (1949) have suggested that the action of cortisone and ACTH, which has a similar action on granulation tissue, is due, at least in part, to "inhibition of reactivity" of the connective tissue.

Baxter, Schiller, Whiteside and Straith (1951) observed atrophic changes in epidermis, collagen fibers, hair follicles and pancreatic adipose on parenteral injection of cortisone. Castor and Baker (1950) observed the same general reaction when cortisone was applied percutaneously to the skin. The latter group further noted that the elastic fibers were not affected.

Seifter et al. (1953) have shown that the adrenal hormones have a direct effect on mucopolysaccharides which are depolymerized by hyaluronidase. They have suggested that this action is probably not an inactivation of the enzyme but an alteration of the substrate.

It may therefore be concluded that in general the adrenal hormones inhibit the rate of healing of wounds and probably do so through their action on the structural element of the tissue.

Gonadal Hormones

There is, at this time, no clear explanation of the effects of the gonadal hormones on the course of healing of wounds. Indeed, there seems to be confusion concerning the action of these hormones on the rate of healing. This confusion may arise partially from the fact that the wounds studied vary as to type and site of infliction. Further confusion is probably introduced by
administration of various doses of the gonadal hormones. This applies to other hormones as well as to the gonadal hormones.

Taubenhaus et. al. (1952) have reported that estradiol inhibited granulation tissue formation in rats, in which turpentine abscesses were induced in the region of the scapulae. Similar results were obtained when the hormone was administered in doses ranging from 0.001 mg. to 1 mg. in a single injection. Sjovall (1947), however, reported favorable results with estradiol benzoate in spayed female rats with vaginal wounds. Brush (1945) reported that stilbestrol did not greatly affect the character of the healing process in fractures of the fibula.

As mentioned previously, serum cholinesterase activity appears to parallel estrogen activity (Sawyer and Everett, 1946). This may be significant, but at the present time the implications of this fact, with relation to wound healing, are not clear.

Generally the same situation is observed regarding the relation of testosterone to wound healing. Early work indicates that testes, either sliced or pulped (Aievoli, 1923; Voronoff and Bostwick, 1918), applied locally to the wounds exerted a favorable influence. Coccarelli (1930) and Lauber (1933) obtained significant shortening of the period required to heal wounds by the administration of testes extract. More recently Abbott et. al. (1946) and Meyer and his coworkers (1947) obtained some indication that testosterone may be of value in the treatment of thermal injuries. However, Taubenhaus and Amromin (1949, 1950) and Taubenhaus et. al. (1952) have observed an inhibition of granulation tissue on the administration of testosterone propionate.
Marsili and Sonetti (1952) using testosterone propionate have obtained results which may help to clarify the situation. When small doses of testosterone propionate was administered either to castrate or normal animals, an acceleration of the cicatrization of the wound was observed. However, large doses produced "scar formation corresponding to toxic phenomena." It seems possible that in the early work where sliced or pulped testes or testes extract was administered, the effective amount of testosterone administered was small and therefore produced an acceleration of healing, while in the latter cases the dose of hormone administered may have been much greater than maximum thus producing an inhibition. The observations of Kochakian et al. (1950) on unwounded, castrated adult rats support this view to some degree. In this experiment low dose levels of testosterone propionate induced the deposition of protein into the carcass, seminal vesicles and prostate, liver and kidney. If the treatment was intensified by the administration of greater doses or by the increased duration of the treatment, the carcass began to lose both protein and fat.

**Pituitary Hormones**

Taubenhaus and Amromin (1950) have reported that the adrenocorticotrophic hormone of the anterior pituitary exhibited an effect on wound healing similar to that exhibited by cortisone, though less pronounced. Baxter, Schiller and Whiteside (1951) have obtained equivocal results with the use of ACTH in human subjects. It seems quite probable that the effect of ACTH would simulate the effect of the adrenal steroids resulting in a decreased production of granulation tissue in the healing wound.
It has been shown that pituitary growth hormone, when administered to rats, produced gigantism (Evans, Simpson, and Li, 1948). This effect might lead one to suspect that growth hormone would increase the rate of deposition of protein and thus increase the rate of healing of wounds. Taubenhaus and Amromin (1950) indicated an increased production of fibroblasts in rats injected with 100 gamma/day of growth hormone, but a failure to stimulate when the dose level was increased to 500 gamma/day. Cuthbertson et. al. (1941), however, failed to observe any significant effect of pituitary extract on the total period required to heal wounds. Since the extract used was a crude alkaline extract, it is possible that ACTH or another material or materials may have been present in the extract in such concentrations as to be antagonistic to the action of the growth hormone. The results may also be explained by the fact that the rate of healing was followed by the wound area method.

The administration of growth hormone to adrenalectomized rats failed to produce a stimulatory effect (Taubenhaus and Amromin, 1950). It would seem then that the effect of growth hormone depends on the presence of the adrenal.

Urinary nitrogen excretion is decreased on administration of growth hormone (Bennett et. al., 1946, Whitney et. al., 1946). Szego and White (1949) report that concurrent with a decreased loss of nitrogen there is an acceleration of fat metabolism. The accumulation of liver lipid in this experiment was independent of the presence of the adrenals, which would seem to indicate that the action of growth hormone on the healing of wounds is not directly related to its action on lipid metabolism.

**Other Hormones**

An increase in thyroid activity was observed by Romani (1952), after
severe scalding of the skin of guinea pigs. Thyroxin has been reported to have no effect on granulation tissue in normal rats (Taubenhaus et. al., 1952), but stimulates granulation tissue formation in hypophysectomized rats (Taubenhaus and Amromin, 1950).

Insulin exerts a favorable influence on the healing process if given before the wound is produced, but has no effect if given after the operation (Scorzotti, 1936). It may promote the synthesis of protein from circulating amino acids (Lotspeich, 1949).

Auerbach (1952) performed an experiment in which two wounds were produced in the same animal in such a manner that the last phase of healing in one wound corresponded with the phase of active proliferation of the second wound. The results obtained show that there is an inhibitory effect during a limited period of wound healing. Auerbach suggests that the experiment may indicate the existence of an inhibiting factor.
CHAPTER III

EXPERIMENTAL PROCEDURE

In the experiments to be described in this section, the effect of the hormone under consideration on both the metabolism and the rate of healing of the wound was studied. The effect on metabolism was studied by following the nitrogen and sulfur balance of the experimental animals. Although a number of authors have investigated the nitrogen balance during the healing of wounds (Meyer, et al., 1947; Abbott, et al., 1946; Bennett, et al., 1946), none have studied the sulfur balance in connection with the effect of hormones in wound healing.

There are a number of advantages for the use of the albino rat as an experimental animal. One of the chief advantages is the fact that they are quite resistant to infection. The animal is easy to handle and requires little space. Furthermore, a large part of the work done in this field has been done using albino rats. Because of these considerations, the Sprague-Dawley strain of rats was chosen as the experimental animal to be used. In all the experiments to be described, the females were used since it was necessary to keep the hormonal environment the same. The animals were inspected for signs of disease before beginning the experiment and were discarded if any such signs were noted. The body weight of the animals was controlled within certain limits for each experiment. The animals were weighed at intervals throughout the experiments.
Twenty-four hour urine samples were collected at intervals during the experiments. The samples were stored under toluene at approximately four degrees centigrade until nitrogen and sulfur analyses could be made.

Total urinary nitrogen was determined by the microkjeldahl technique. In this procedure a 1 cc. aliquot of urine was digested with concentrated sulfuric acid for forty-five minutes, after which 5 drops of 30% hydrogen peroxide was added and the digestion continued for an additional fifteen minutes. The nitrogenous material is converted to ammonia by this procedure. The ammonia is trapped on formation by conversion to ammonium ions in the acid medium. After digestion the material is transferred to a microkjeldahl apparatus, the solution made basic with concentrated sodium hydroxide and the ammonia steam distilled into a standardized sulfuric acid solution. The remaining acid is back titrated to a methyl red endpoint with standardized sodium hydroxide.

Total sulfur was determined by the combined methods of Masters (1939) and Treon and Crutchfield (1942). An aliquot of urine is digested with nitric and perchloric acid mixture (3:1 by volume). The sample was then evaporated to a wet ash and treated with 30% hydrogen peroxide. The procedure converts sulfur to sulphate which can then be determined turbidimetrically in the Klett-Sommerson colorimeter.

The animals were acclimated to the diet and environment for approximately five days, depending on the experiment, before they were wounded. The rats were anesthetized with nembutal (2.5 mg/100 gm body weight) injected intraperitoneally. The hair on the back of the neck was removed. A standard three cm. incision was then made in the mid-dorsal portion of this region down
to the muscle. In no case was the muscle injured. The edges of the wound are
apposed and held in position with three evenly spaced 11 cm. Michel wound clips.
After three days, the wound clips were removed. At approximately weekly
intervals one-third of the animals were sacrificed and 0.5 cm. sections of the
wound tissue removed for wound strength determinations. The apparatus is shown
diagrammatically in Figure 2.

The section of tissue is placed in the clamps (a). Pressure is
exerted on the mercury reservoir from the tank of CO₂ (b) forcing the mercury
into the plastic container (c). The increasing stress subsequently causes the
wound tissue to rupture. The fall of the container opens a stopcock (d) which
releases the pressure in the system and stops the flow of mercury. The
container system is then weighed. That weight which causes the wound to rupture
is taken as the wound strength. It is assumed that the cross sectional area
of the wound is the same in all cases.

The hormones which were studied in this investigation were testos-
terone, estrone, thyrotropin, and growth hormone. The testosterone used was
obtained from Eli Lilly and Co. in aqueous suspension (25 mg/cc.). The animals
received 1 mg. six days before wounding, 5 mg. on the day of wounding and 5 mg.
eight days after wounding. The estrone was also obtained from Eli Lilly and Co.
in aqueous suspension (5 mg./cc.). The animals received 1 mg. on the 6th day
before wounding, 1 mg. on the day of wounding, and 1.4 mg. on the eighth day
after wounding. These hormones were injected intramuscularly.

Growth hormone and thyrotropin were obtained from Armour Research
Laboratories. The thyrotropin was injected intramuscularly (0.25 cc. of a
saline solution of 1 USP units/cc.) two days before wounding, on the day of
Fig. 2. Diagrammatic representation of apparatus used to measure wound strength.
wounding and on the 3rd, 8th, 10th and 13th days after wounding. In the experiment using growth hormone, the rats received intraperitoneally either 1.0 or 0.3 mg. of the hormone in 0.15 M Na Cl divided into two daily doses, beginning the day before wounding and continuing throughout the experiment.

In each experiment the animals were offered 8 gms. of basal diet per day. It was found that the animals completely consumed this amount of diet in 24 hours. The composition of the basal diet is shown in Table I. The diet used will be indicated in the discussion of the experiment. Distilled water was given ad libitum.
<table>
<thead>
<tr>
<th>Dietary Component</th>
<th>gm/kgm Diet</th>
<th>Vitamin Supplement</th>
<th>mg/kgm Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>30</td>
<td>Thiamine Hydrochloride</td>
<td>10</td>
</tr>
<tr>
<td>Lard</td>
<td>120</td>
<td>Riboflavin</td>
<td>10</td>
</tr>
<tr>
<td>Salt Mixture (Hubble, et al., 1947)</td>
<td>50</td>
<td>Pyridoxine Hydrochloride</td>
<td>10</td>
</tr>
<tr>
<td>Sucrose</td>
<td>800</td>
<td>Calcium Pantothenate</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nicotinic Acid Amide</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inositol</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Para-amino Benzoic Acid</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-Methylnapthaquinone</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Choline Chloride</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vitamin A (from Oleum Percomorphum)</td>
<td>2500 IU</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vitamin D (from Oleum Percomorphum)</td>
<td>360 IU</td>
</tr>
</tbody>
</table>
CHAPTER IV

RESULTS AND DISCUSSION

The general plan of the experiments which were performed in this study was discussed in the previous section of the thesis. In this chapter the results of the experiments are presented and their significance discussed.

The Effect of Gonadal Hormones:

In experiment 1, the effect of sex hormones on the rate of healing of experimental wounds was investigated. The 63 rats used in this experiment were acclimated to laboratory conditions for six days before being wounded. The animals were maintained on a 3% casein diet (Table I) throughout the experiment. The average body weight of the rats at the beginning of the experiment was 166.20 gms. On the day of wounding, however, the group of animals receiving estrone had lost about 14 grams while the controls and testosterone-treated animals showed no significant change in weight. The body weight change from the day of wounding is plotted against time after wounding in Figure 3 in order that the weight change after the wounding procedure be comparable among the groups. From this graph it may be seen that the body weight change after the wounding procedure was not significantly different between the controls and the groups receiving estrone (21 rats) or testosterone (21 rats).

It was noted in a previous section of this thesis that when the strength of healing wounds (wound strength) is plotted against time, a curve
Fig. 3. The effect of gonadal hormones on the body weight loss after wounding. Closed circles—control; half closed circles—testosterone treated; open circles—estrone treated.
very similar to a growth curve is obtained (Figure 1). The portion of this curve between approximately the 6th and 20th days after wounding is essentially a straight line. This corresponds to the portion of the curve between points A and B in Figure 1. This line may be described by the equation:

\[ y = kx + b \]

where \( k \) is the slope of the line and \( b \) is the intercept on the y axis. Differentiating the equation we obtain:

\[
\frac{dy}{dx} = k
\]

That is, the rate of change of \( x \) with respect to \( y \) is equivalent to the slope of the line and is a constant. It then follows that the slope of the wound strength versus time curve is a numerical evaluation of the rate of change of tensile strength with respect to time. This constant has been called the healing index (Williamson and Fromm, 1952, 1953; Fromm, 1952) and may be considered to be a measurable function of the rate of healing of wounds.

The healing indices were determined for the wounds of the animals used in this experiment. At approximately weekly intervals after wounding, \( 1/3 \) of the animals of each group were sacrificed. 0.5 cm. sections of the healing wounds of these animals were excised for wound strength determinations. The results are shown in Table II and are plotted in Figure 4. On the nineteenth day after wounding, the wound strengths of the wounds in the control rats were significantly greater than those of the wounds in the hormone treated animals. This indicates that the total period required to heal the wound had been lengthened because of the administration of the gonadal hormones. The healing index for the wounds in the control animals was greater than that of the wounds
# TABLE II

## THE EFFECT OF GONADAL HORMONES ON THE WOUND STRENGTH OF EXPERIMENTAL WOUNDS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days After Wounding</th>
<th>Healing Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>Control</td>
<td>$195 \pm 9$</td>
<td>$400 \pm 24$</td>
</tr>
<tr>
<td>Estrone</td>
<td>$163 \pm 7$</td>
<td>$287 \pm 13$</td>
</tr>
<tr>
<td>Testosterone</td>
<td>$195 \pm 15$</td>
<td>$326 \pm 16$</td>
</tr>
</tbody>
</table>

"p" values: on the nineteenth day after wounding.

"p" between control and estrogen less than 0.01.

"p" between control and testosterone less than 0.01.
Fig. 4. The effect of gonadal hormones on the rate of healing of wounds. Open circles—controls; half closed circles—testosterone treated; closed circles—estrone treated.
in either of the experimental groups of animals.

Williamson and Fromm (1952) and Fromm (1952) have shown that the urinary N/S ratio may be correlated to the healing index. In their experiments it was noted that the animals exhibiting the greatest rate of healing of the wounds also exhibited the greatest urinary N/S ratio. The increased nitrogen to sulfur ratio was taken to indicate a proportionately greater retention of sulfur by the animals exhibiting the greatest healing index as compared to animals exhibiting a lower healing index. It was thought that this situation was a reflection of a more rapid rate of incorporation of sulfur into the wounds of the animals exhibiting the greatest rate of healing.

Determination of the nitrogen and sulfur balances and the urinary N/S ratio show no differences in these quantities between the various groups of animals in this experiment (Table III). The apparent differences between the present work and that of Williamson and Fromm may be due to the difference in the hormonal environment in the experimental animals. It is known that the sex hormones affect protein metabolism and show "Tissue specificity". For example, testosterone will cause a loss of fat and protein from the carcass of an animal when given in large doses. The hormone simultaneously causes the deposition of protein in the seminal vesicles, prostate, kidney and liver of male castrate rats (Kochakian et. al., 1950).

This "tissue specificity" of testosterone may possibly be responsible for its effect on the rate of healing of wounds. This effect may also explain the similar values for the nitrogen and sulfur balance between the rats receiving testosterone and the control rats. A similar mode of action may be responsible for the effect of estrone on the rate of healing of experimental wounds.
### TABLE III

THE EFFECT OF GONADAL HORMONES ON THE AVERAGE N AND S BALANCE AND THE URINARY N/S RATIO DURING THE HEALING OF EXPERIMENTAL WOUNDS

<table>
<thead>
<tr>
<th></th>
<th>N Output (mg.)</th>
<th>N Intake (mg.)</th>
<th>N Balance (mg.)</th>
<th>S Output (mg.)</th>
<th>S Intake (mg.)</th>
<th>S Balance (mg.)</th>
<th>N/S Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>73.3</td>
<td>32.9</td>
<td>-40.4</td>
<td>3.1</td>
<td>1.2</td>
<td>-1.9</td>
<td>23.5</td>
</tr>
<tr>
<td>Estrone</td>
<td>79.6</td>
<td>31.5</td>
<td>-48.8</td>
<td>3.8</td>
<td>1.2</td>
<td>-2.7</td>
<td>23.0</td>
</tr>
<tr>
<td>Testosterone</td>
<td>75.3</td>
<td>32.9</td>
<td>-43.1</td>
<td>3.0</td>
<td>1.2</td>
<td>-1.8</td>
<td>26.9</td>
</tr>
</tbody>
</table>
From the data cited above, it is concluded that the gonadal hormones inhibit the rate of healing of the wound. Even though the hormones used inhibit the rate of healing, the nitrogen and sulfur excretion is not noticeably different in the experimental animals as compared to the controls.

The Effect of Thyrotropin:

Experiment 2 was designed to test the effect of pituitary thyrotropic hormone (TH) on the rate of healing of experimental wounds. The 42 animals in this experiment were acclimated to the laboratory conditions and to the diet for a period of six days. A solution of thyrotropin was administered to 21 rats as previously described. The remaining animals were injected with an equal volume of isotonic saline. The weight of the rats at the beginning of the experiment was approximately 199.20 gms. The weight data are shown graphically in Figure 5. The continued loss of weight by both groups of rats is reflected in a negative nitrogen balance. The TH treated animals lost more weight and retained less nitrogen than the controls (Figure 6).

Wound strength determinations were made at approximately weekly intervals after wounding (Table IV). The results indicate that the administration of thyrotropin prolongs the time necessary to bring the wound strength to the same level as that attained by the wounds of the control animals. The data is plotted in Figure 7. On the 19th day after wounding, the wound strength of the wounds in the control rats was significantly greater than that of the test wounds. The statistical significance is indicated in Table IV.

It will be noticed that the healing index for the wounds in the TH treated group is only very slightly less than that of the controls. The lower wound strength of the test wounds on the 7th day after wounding suggests that
Fig. 5. The effect of thyrotropin on body weight during the healing of wounds. Open circles - control; closed circles - thyrotropin treated.
Fig. 6. The effect of thyrotropin on the nitrogen balance during the healing of wounds. Open circles—control; closed circles—thyrotropin treated.
TABLE IV

THE EFFECT OF THYROTROPIN ON THE WOUND STRENGTH OF HEALING WOUNDS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days After Wounding</th>
<th>Healing Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>212 ± 18</td>
</tr>
<tr>
<td>Thyrotropin</td>
<td>7</td>
<td>179 ± 19</td>
</tr>
</tbody>
</table>

"p" less than 0.01 on the 19th day after wounding.
Fig. 7. The effect of thyrotropin on the rate of healing of wounds. Open circles - control; closed circles - thyrotropin treated.
the "lag" period has been lengthened. The "lag" period is that period during which the wound strength changes of the wound cannot be detected by the apparatus used in these experiments. In addition to this there is some indication that the rate of increase in wound strength is speeded during the earlier portion of the progressive phase of healing, by the administration of the TH, but inhibited during the later stages. Since the healing index is the average slope over the entire period of the experiment, these considerations may account for the similarity in the healing indices of the two groups.

This situation may be a reflection of the fact that the TH has stimulated the production of thyroid hormone only slightly in the earlier stages of healing, but more vigourously in the later stages. Koger, Hurst and Turner (1942) have obtained data which indicate that there is a dose level of thyroxine which causes a maximal increase in body weight. Doses above this level apparently are less efficient in producing an increase in weight and may even cause weight decreases. Taubenhaus, et. al. (1952) have reported that thyroxine has no effect on the granulation tissue of wounds in normal animals, but does stimulate granulation tissue formation in hypophysectomized rats.

It might be suggested, therefore, that the TH stimulated the production of thyroid hormone slightly during the first part of the experiment. This may have resulted in a small increase in metabolic rate and a relatively more rapid rate of healing. In the later part of the experiment, however, TH may have stimulated the production of thyroid hormone to such an extent that the increased metabolic rate inhibited the rate of healing. This situation may have led to the overall inhibition of healing evidenced by the difference in wound strengths on the 19th day after wounding.
The Effect of Growth Hormone:

Since the pituitary growth hormone (GH) is known to stimulate growth (Evans et al., 1948; Whitney et al., 1948), nitrogen retention (Bennett et al., 1946; Whitney et al., 1948), and sulfur retention (Gaebler and Price, 1937) it was thought that administration of this hormone might stimulate the healing of experimental wounds. Experiments were undertaken to test this hypothesis.

Gordan et al. (1948) have shown that the level of dietary protein affects the action of growth hormone on nitrogen retention and growth. This work has shown that the higher the protein content of the diet, the greater the growth rate and the greater the nitrogen retention. When 1 mg/day of GH was administered to rats fed a 6% casein diet, the animals exhibited a slight increase in growth rate and a slightly more positive nitrogen balance as compared to their controls. No data were available for the effect of GH (1mg/day) on the nitrogen balance of rats fed a 3% protein diet. Since GH may have no effect on the nitrogen balance when the level of dietary protein is 3%, it was decided to increase the protein content of the diet used in the present study. However, since it was desired to keep the nitrogen intake of the rats at a low value, the protein content of the diet was increased only to 6%. Thus, the diet used in the two experiments to be described contained 60 gms. of casein and 770 gms. of sucrose per kilogram of diet; the remainder of the diet was the same as indicated in Table I.

In Experiment III, 0.5 mg. GH in saline was administered twice daily to 32 of the 64 rats in the experiment. The control group received an equivalent volume of isotonic saline. At the beginning of the experiment, the
average weight of all the animals was 200 20 gms. On completion of the experiment it was noted that the weight loss of the rats treated with GH was significantly less than that of the control animals (Figure 8). The loss of weight exhibited by the controls may be expected since wounded rats normally lose weight under the conditions of the experiment. In would appear, then that the administration of 1 mg/day of GH prevents the loss of body weight by wounded animals.

The weight changes exhibited by the two groups of rats are reflected in the nitrogen and sulfur balances of the animals. The GH treated rats were in a more positive nitrogen and sulfur balance than were the control rats (Tables V and VI). The differences in the average nitrogen and sulfur balance between the two groups were significantly different ("p" values less than 0.001 for both nitrogen and sulfur). The average urinary N/S ratio for the rats receiving GH was greater than that exhibited by the control animals (Figure 9). This difference, however, did not become apparent until after about the 7th day after wounding.

Despite the fact that the animals treated with GH lost less weight and exhibited a greater nitrogen and sulfur retention than did the control rats, the wound strength of the wounds of the treated animals was significantly less than that of the wounds of the control animals on the 19th day after wounding (Table VII). In Figure 10 the wound strength is plotted against time after wounding. It can be seen from this graph that the wounds of the GH treated animals healed at an appreciably slower rate than those of the control rats. This is also apparent in the values for the healing indices (Table VII).

The work of Williamson and Fromm (1952) has already been referred to
TABLE V

THE EFFECT OF GROWTH HORMONE (1 mg/day) ON THE NITROGEN BALANCE DURING THE HEALING OF EXPERIMENTAL WOUNDS

<table>
<thead>
<tr>
<th>Day After Wounding</th>
<th>Control</th>
<th></th>
<th></th>
<th>GH Treated</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Output</td>
<td>Intake</td>
<td>Balance</td>
<td>Output</td>
<td>Intake</td>
<td>Balance</td>
</tr>
<tr>
<td>1</td>
<td>81.8</td>
<td>65.8</td>
<td>-16.0</td>
<td>68.2</td>
<td>65.8</td>
<td>-4.3</td>
</tr>
<tr>
<td>3</td>
<td>55.4</td>
<td>65.8</td>
<td>10.4</td>
<td>33.7</td>
<td>65.8</td>
<td>24.5</td>
</tr>
<tr>
<td>4</td>
<td>71.4</td>
<td>65.8</td>
<td>-5.6</td>
<td>42.0</td>
<td>65.8</td>
<td>21.7</td>
</tr>
<tr>
<td>7</td>
<td>67.2</td>
<td>65.8</td>
<td>-1.4</td>
<td>75.9</td>
<td>65.8</td>
<td>-10.1</td>
</tr>
<tr>
<td>10</td>
<td>74.9</td>
<td>65.8</td>
<td>-9.1</td>
<td>55.2</td>
<td>65.8</td>
<td>10.6</td>
</tr>
<tr>
<td>13</td>
<td>67.6</td>
<td>65.8</td>
<td>-1.8</td>
<td>50.8</td>
<td>65.8</td>
<td>15.0</td>
</tr>
<tr>
<td>15</td>
<td>66.3</td>
<td>65.8</td>
<td>-0.5</td>
<td>64.4</td>
<td>65.8</td>
<td>1.4</td>
</tr>
<tr>
<td>17</td>
<td>59.3</td>
<td>65.8</td>
<td>6.3</td>
<td>57.8</td>
<td>65.8</td>
<td>8.0</td>
</tr>
<tr>
<td>Average</td>
<td>68.0</td>
<td>65.8</td>
<td>-2.2</td>
<td>56.0</td>
<td>65.8</td>
<td>8.3</td>
</tr>
</tbody>
</table>
TABLE VI

THE EFFECT OF GROWTH HORMONE (1 mg/day)
ON THE SULFUR BALANCE DURING
THE HEALING OF EXPERIMENTAL WOUNDS

<table>
<thead>
<tr>
<th>Day After Wounding</th>
<th>Control</th>
<th>GH Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Output</td>
<td>Intake</td>
</tr>
<tr>
<td>1</td>
<td>3.5</td>
<td>3.2</td>
</tr>
<tr>
<td>3</td>
<td>3.6</td>
<td>3.2</td>
</tr>
<tr>
<td>4</td>
<td>4.0</td>
<td>3.2</td>
</tr>
<tr>
<td>7</td>
<td>4.4</td>
<td>3.2</td>
</tr>
<tr>
<td>10</td>
<td>2.0</td>
<td>3.2</td>
</tr>
<tr>
<td>13</td>
<td>2.2</td>
<td>3.2</td>
</tr>
<tr>
<td>15</td>
<td>2.4</td>
<td>3.2</td>
</tr>
<tr>
<td>17</td>
<td>2.4</td>
<td>3.2</td>
</tr>
<tr>
<td>Average</td>
<td>2.9</td>
<td>3.2</td>
</tr>
</tbody>
</table>
Fig. 8. Body Weight change after wounding. Open circles—controls; closed circles—GH (1 mg/day).
Fig. 9. Urinary N/S ratio during the healing of wounds. Open circles represent control values, closed circles represent urinary N/S ratios in animals treated with 1 mg/day GH.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days After Wounding</th>
<th>Healing Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7 13 19 k</td>
<td></td>
</tr>
<tr>
<td>Growth Hormone (1 mg/day)</td>
<td>191±2 451±8 761±16</td>
<td>47.4</td>
</tr>
<tr>
<td></td>
<td>187±3 386±17 638±21</td>
<td>37.6</td>
</tr>
</tbody>
</table>

"p" on the nineteenth day less than 0.01.
Fig. 10. Effect of Growth Hormone on Rate of Healing of Wounds. Open Circles—control; closed circles—GH (1 mg/day).
in the discussion of Experiment I of this study. It will be remembered that these authors have shown that those rats, in which the wounds heal most rapidly, exhibit the greatest urinary N/S ratio. However, the results obtained in this study, in both Experiment I and Experiment III, show that this generalization does not, necessarily, apply to those animals with an abnormal hormonal environment.

Taubenhaus and Amromin (1950) have reported a stimulation of granulation tissue formation on administration of 0.1 mg/day of GH, but noted that 0.5 mg/day of GH had no apparent effect. In order to check this apparent reversal of effect on increasing the dose of the hormone, an experiment (IV) was performed. Experiments III and IV were identical except for the dose of Hormone used. In Experiment IV, 0.15 mg. GH was administered twice daily to 1/3 of the animals of the experiment.

In contrast to Experiment III, the wounds of the treated animals in Experiment IV showed a significantly greater wound strength on the 19th day after wounding than did the wounds of the control rats (Table VIII). The wound strength data is plotted in Figure 11. The results show that when GH is given at relatively low dose levels the hormone stimulates the healing of the wound, as compared to simultaneous controls. On the other hand, large doses of GH decreases the rate of healing, as had been shown in Experiment III.

In Experiment IV, as in Experiment III, the weight loss of the treated animals was significantly less than that of the control rats (Figure 12). If it is assumed that the differences in weight loss, nitrogen balance and sulfur balance between the control group and the treated group in Experiments III and IV is due only to the hormone, it is then possible to compare the
TABLE VIII

THE EFFECT OF GROWTH HORMONE (0.3 mg/day) ON THE WOUND STRENGTH OF HEALING WOUNDS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days After Wounding</th>
<th>Healing Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>148 ± 4</td>
<td>329 ± 6</td>
</tr>
<tr>
<td>Growth Hormone (0.3 mg/day)</td>
<td>162 ± 6</td>
<td>292 ± 7</td>
</tr>
</tbody>
</table>

"p" on the nineteenth day less than 0.01.
Fig. 11. Effect of growth hormone on the rate of healing of wounds. Open circles—control; closed circles—GH (0.3 mg/day).
Fig. 12. The effect of growth hormone on body weight loss after wounding. Open circles—control; closed circles—growth hormone, (0.3 mg/day).
effects of the dose levels of the GH on these quantities. The results of such a calculation are shown in Table IX. When the weight "conservation" caused by the two dose levels of hormone are thus compared, it is seen that the lower doses of GH causes a smaller "conservation" of weight than the larger dose, as might be expected. However, the smaller dose of GH appears to be the more efficient in conserving the body weight; i.e. the ratio of body weight conserved to the total dose of hormone administered is larger for the lower dose than for the larger dose of GH. For the dose level of 1 mg/day this ratio is 0.53, while for the dose level of 0.3 mg/day the ratio is 0.93.

In Experiment IV the control animals exhibited a significantly more negative nitrogen balance (Table X) and a significantly more negative sulfur balance (Table XI) than did the GH treated rats. When the nitrogen conservation caused by the GH is compared, in the same manner as the weight changes, it is seen that the nitrogen retention reflects the changes in weight. The greater dose of GH causes a conservation of more nitrogen than the smaller dose, but appears to be less efficient. Thus, the administration of 1 mg/day of GH causes the retention of 10.5 mg. N/mg. GH, while administration of 0.3 mg/day of GH causes the retention of 19.3 mg. N/mg. GH.

However, when the sulfur balance is considered (Table IX), it appears that the smaller quantity of GH is about four times as efficient as the larger quantity in inhibiting the loss of sulfur, since both dose levels cause the same conservation of sulfur by the wounded animal. This would indicate that when 0.3 mg/day of GH is administered to the animal, sulfur is more readily available to the regenerating wound tissue than when 1 mg/day of the hormone is administered. This interpretation is further supported by the fact that 1
### TABLE IX

**THE EFFECT OF GROWTH HORMONE ON BODY WEIGHT LOSS AND NITROGEN AND SULFUR CONSERVATION DURING THE HEALING OF EXPERIMENTAL WOUNDS: COMPARISON OF DOSE LEVELS**

<table>
<thead>
<tr>
<th></th>
<th>Control (Expt.III)</th>
<th>GH (1 mg/day)</th>
<th>Control (Expt.IV)</th>
<th>GH (0.3 mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body Weight Loss</strong></td>
<td>16.0</td>
<td>5.9</td>
<td>14.4</td>
<td>9.1</td>
</tr>
<tr>
<td><strong>Conservation of Body Weight due to GH</strong></td>
<td>10.1</td>
<td></td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td><strong>Average N Balance</strong></td>
<td>-2.2</td>
<td>8.3</td>
<td>-13.3</td>
<td>-7.5</td>
</tr>
<tr>
<td><strong>Nitrogen Conservation due to GH</strong></td>
<td>10.5</td>
<td></td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td><strong>Average S Balance</strong></td>
<td>0.3</td>
<td>0.7</td>
<td>-0.4</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Sulfur Conservation due to GH</strong></td>
<td>0.4</td>
<td></td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td><strong>Urinary N/S due to GH</strong></td>
<td>16:1</td>
<td></td>
<td>11.6:1</td>
<td></td>
</tr>
</tbody>
</table>
### TABLE X

**THE EFFECT OF GROWTH HORMONE (0.3 mg/day) ON THE NITROGEN BALANCE DURING THE HEALING OF EXPERIMENTAL WOUNDS**

<table>
<thead>
<tr>
<th>Day After Wounding</th>
<th>Control</th>
<th>GH Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Output</td>
<td>Intake</td>
</tr>
<tr>
<td>1</td>
<td>98.5</td>
<td>65.8</td>
</tr>
<tr>
<td>4</td>
<td>73.4</td>
<td>65.8</td>
</tr>
<tr>
<td>6</td>
<td>80.4</td>
<td>65.8</td>
</tr>
<tr>
<td>8</td>
<td>77.6</td>
<td>65.8</td>
</tr>
<tr>
<td>11</td>
<td>84.9</td>
<td>65.8</td>
</tr>
<tr>
<td>14</td>
<td>76.3</td>
<td>65.8</td>
</tr>
<tr>
<td>17</td>
<td>79.8</td>
<td>65.8</td>
</tr>
<tr>
<td>19</td>
<td>62.2</td>
<td>65.8</td>
</tr>
<tr>
<td>Average</td>
<td>79.1</td>
<td>65.8</td>
</tr>
</tbody>
</table>
TABLE XI

THE EFFECT OF GROWTH HORMONE (0.3 mg/day) ON THE SULFUR BALANCE DURING THE HEALING OF EXPERIMENTAL WOUNDS

<table>
<thead>
<tr>
<th>Day After Wounding</th>
<th>Control Output</th>
<th>Control Intake</th>
<th>Control Balance</th>
<th>GH Treated Output</th>
<th>GH Treated Intake</th>
<th>GH Treated Balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.4</td>
<td>3.2</td>
<td>-2.2</td>
<td>4.6</td>
<td>3.2</td>
<td>-1.4</td>
</tr>
<tr>
<td>4</td>
<td>3.1</td>
<td>3.2</td>
<td>0.1</td>
<td>2.6</td>
<td>3.2</td>
<td>0.6</td>
</tr>
<tr>
<td>6</td>
<td>3.4</td>
<td>3.2</td>
<td>0.2</td>
<td>3.3</td>
<td>3.2</td>
<td>-0.1</td>
</tr>
<tr>
<td>8</td>
<td>3.3</td>
<td>3.2</td>
<td>-0.1</td>
<td>3.2</td>
<td>3.2</td>
<td>0.0</td>
</tr>
<tr>
<td>11</td>
<td>3.6</td>
<td>3.2</td>
<td>-0.4</td>
<td>3.1</td>
<td>3.2</td>
<td>0.1</td>
</tr>
<tr>
<td>14</td>
<td>3.7</td>
<td>3.2</td>
<td>-0.5</td>
<td>3.7</td>
<td>3.2</td>
<td>-0.5</td>
</tr>
<tr>
<td>17</td>
<td>3.2</td>
<td>3.2</td>
<td>0.0</td>
<td>2.1</td>
<td>3.2</td>
<td>1.1</td>
</tr>
<tr>
<td>19</td>
<td>3.4</td>
<td>3.2</td>
<td>-0.2</td>
<td>2.2</td>
<td>3.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Average</td>
<td>3.6</td>
<td>3.2</td>
<td>-0.4</td>
<td>3.1</td>
<td>3.2</td>
<td>0.1</td>
</tr>
</tbody>
</table>
mg/day of GH causes the retention of nitrogen and sulfur in the ratio of 16:1, but 0.3 mg/day causes this ratio to be decreased to about 12:1 (Table IX). In other works, the administration of 1 mg/day of GH causes the conservation of these elements (N and S) in the ratio which one would expect if these materials were being deposited into normal body proteins. However, administration of 0.3 mg/day of GH causes the conservation of nitrogen and sulfur in a ratio which may indicate the deposition of a greater proportion of material of high sulfur content. These considerations would, therefore, seem to indicate that large amounts of GH mobilize nitrogen and sulfur primarily for use by the body tissues to the detriment of the wound tissue. Smaller amounts of the GH permit a greater diversion to the regenerating tissue.

The data obtained in these experiments do not exclude the possibility that large doses of GH may suppress the activity of another factor(s) which may be necessary for the normal healing of the wound. In this connection it is interesting to note again the work of Taubenhaus and Amromin (1950) who have reported a failure to observe the stimulatory action of the pituitary growth hormone on the wound tissue in the absence of the adrenal glands. It would seem, then, that the adrenal gland is somehow involved in the action of GH on the healing wound.
CHAPTER V

SUMMARY AND CONCLUSIONS

The effect of certain hormones on the rate of healing of experimental wounds was investigated. The metabolism of nitrogen and sulfur during the healing of wounds was studied simultaneously.

The rate of healing of standard wounds in female albino rats was determined by the wound strength method. The animals were fed a basal diet of known nitrogen and sulfur content. The urinary excretion of these elements were determined. Appropriate controls were used in each experiment.

The results of these studies indicate that:

1. Testosterone and estrone inhibit the rate of healing of the wound under the conditions of the experiment. These hormones apparently did not affect the nitrogen and sulfur balances of the wounded animals. The body weight loss of the wounded rats also seemed not to be affected by the injection of these hormones.

2. The period required for the healing of the wound was lengthened by the injection of the pituitary thyrotropic hormone. The rats treated with TH exhibited a greater loss of weight and a more negative nitrogen balance than the control animals.

3. The administration of GH causes a conservation of body weight in wounded animals and a parallel conservation of nitrogen. Under the conditions
of the experiments, a relatively low dose of GH (0.3 mg/day) appears to be about
twice as efficient in conserving body weight and nitrogen in wounded animals
than does a greater dose (1.0 mg/day). The smaller dose of the hormone
appears to be about four times as efficient in conserving sulfur than the
larger dose.

4. The administration of 1 mg/day of GH caused a decreased rate of
healing of the wound, while 0.3 mg/day caused an increased rate of healing, as
compared to the respective controls.

5. It was observed that the ratio of conserved nitrogen to conserved
sulfur was about 16:1 for those animals receiving 1 mg/day of GH, but about
12:1 for those animals receiving 0.3 mg/day. These ratios suggest that the
administration of GH in large doses mobilizes nitrogen and sulfur primarily for
use by normal body tissues to the detriment of the wound. Smaller amounts,
however, allow greater diversion of nitrogen and sulfur to the wound tissue.
BIBLIOGRAPHY


Barnes, T.C. (1945), "Healing Rate of Human Skin Determined by the Measurement of Electrical Potential of Experimental Abrasions; Study of Treatment with Petrolatum and with Petrolatum Containing Yeast and Liver Extracts," Am. J. Surg. 69: 82-88.


APPROVAL SHEET

The thesis submitted by George J. Neumann has been read and approved by three members of the faculty of the Stritch School of Medicine, Loyola University.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

Date 7/1/54

Signature of Adviser