Although the problem of the healing of wounds has commanded much interest for a long time, the metabolic changes which accompany wound healing have been seriously investigated only since the early work of Howes and his associates (1–4) has appeared. Much of the literature in this field has been concerned with an empirical approach to the acceleration of the healing processes. The lack of quantitatively precise methods for measuring the rates of healing, even in experimental wounds, has impaired the value of much of this work. Nonetheless, a number of metabolic implications can be gleaned from these empirical studies. Only relatively recently have systematic studies been undertaken on metabolism after injury.

The biochemical work on wounds and wound healing has been devoted largely to the metabolism of proteins by the injured organism and the determination of the character and metabolism of certain proteins and polysaccharides of the wound area. The latter have been of interest because of their close association with the protein fibers found not only in wound tissue but also in connective tissue in general. The present discussion will be limited to the consideration of some aspects of the metabolism of proteins, and their constituent amino acids, which are influenced by the infliction of various types of trauma.

**NITROGENOUS CONSTITUENTS OF THE BLOOD**

Disturbances in protein metabolism are usually reflected in changes of the level of the various nitrogen-containing compounds found in the different fractions of the blood. Since protein metabolism is known to
be profoundly affected after trauma, it is not unexpected that changes in the level of the plasma constituents have been observed during the healing of wounds.

**Plasma Proteins**

The studies on the total plasma proteins have not always yielded consistent results (5, 6). These inconsistencies are probably attributable to two principal causes: (1) the differences in severity of injury considered in different reports and (2) the possibility that the concentration of some fractions of the plasma proteins might be increasing while others are decreasing. The latter has been definitely implicated as one reason for the inconsistencies observed, although the former has not been ruled out entirely. By the use of electrophoretic technics, the changes in the levels of the plasma protein fractions have been clearly and repeatedly demonstrated.

**Albumin**

Data from a number of laboratories have indicated that there is a small drop in the level of the plasma albumin directly after injury (7–12, 109). The decrease in the plasma albumin concentration appears to be in the neighborhood of 10–20 per cent of the pretraumatic level. All of these experiments were carried out on animals in which the quantity of plasma proteins prior to wounding might be considered to be in the normal range. Since it has been shown that in the presence of a low plasma protein level the rate of healing is much inhibited (19, 38), it would be of interest to know whether injury would result in a further decrease in the albumin level when hypoproteinemia is present before the trauma is made.

**Globulin and Lymph Proteins**

Accompanying the decrease in plasma albumin is a concomitant rise in the total protein content of the lymph. This increase is due not only to an increase in the amount of albumin, but also of the globulins as well (7, 10). Evidently the permeability characteristics in the injured area are altered so that the plasma proteins may pass into the lymph more easily. This effect is particularly evident when the trauma is a burn. The proteins in the lymph, of course, are more readily available for utilization in the regenerative and reparative processes connected with the healing of the trauma. Perlmann et al. (7) indicated that after injury a new protein appears in the lymph with an electrophoretic mobility even lower than that of the γ-globulins at pH levels above 7.0.
It would seem quite probable that this new protein in the lymph arises from the injured tissues. In these studies (7, 8, 9, 12) there was also noted a sharp increase in the α-globulins after injury, while the levels of the β- and the γ-globulins remained essentially unchanged from the normal.  

A most careful and extensive study on the changes in the plasma protein levels after various injuries was carried out by Chanutin and his co-workers (13–16). They fractionated the plasma from normal rats and from those which were injured in various ways, using the alcohol–low temperature technic described by Cohn et al. (17), before determining the amounts of the different plasma proteins in their fractions by electrophoresis. The results they obtained were quite similar to those previously noted—that is, a decrease in albumin, a marked increase in the α-globulins (and particularly in the α1-globulins), and no significant change in the other globulin fractions. The concentration of the plasma proteins seemed to return to approximately normal values in about 10–15 days after the injuries were made. Among other items, they noted that after fractionation the plasma from the injured rats showed new spikes in the electrophoretogram of the α- and the β-globulins. These results may be interpreted to mean that either that new proteins appear in the plasma after injury or that some component of the α- and the β-globulin complexes increases markedly after injury.

**Lipoproteins**

The presence of most, if not all, of the serum lipoproteins in the α-globulin and β-globulin fractions has stimulated the study of these components of the plasma of injured animals (12, 18). These reports indicate that after injury there is a small rise, which may not be significant, in the plasma lipoproteins having a low S; while those having a high SI increase about two or three times over that found in the plasma of normal animals. The implications of these changes in the lipoprotein levels is obscure.

**Fibrinogen**

The fibrinogen content of the plasma has been shown to increase very markedly after injuries (8, 20, 21). This increase reaches a peak of about 70 per cent above normal levels in 1–2 days after injury and then gradually returns to normal over the course of several weeks. Although the

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1 It has been pointed out that these changes in plasma protein levels appear to be relatively nonspecific since they appear in all situations where there is inflammation or destruction of tissue, regardless of cause (8).
clotting time of the blood appears to remain normal, the blood prothrombin level has been reported to be somewhat lower than normal shortly after injury (21).

Hemoglobin

It has also been shown that there is a decrease in the hemoglobin content of the blood after burns (8, 22, 23, 24). It is well established that a low or decreasing hemoglobin level usually acts as a stimulus for the production of more hemoglobin. In spite of the expected stimulation of the low hemoglobin levels, studies on the synthesis of heme with N14-labeled glycine in injured animals indicated that there was a marked depression in the formation of hemoglobin for as long as 25 days after injury. The excretion of increased amounts of urobilinogen and coproporphyrin by the experimental animals during these studies (24) suggests the possibility of an increased destruction of hemoglobin as well.

Blood Enzymes

The results of investigations on the level of several enzymes in the blood after injuries have also been reported. It has been shown that the plasma lipase level decreases after burns, but returns to normal levels upon recovery (25). After severe injuries the carbonic anhydrase of the blood was reported to be much decreased. Slighter injuries caused no detectable depression in the level of the blood carbonic anhydrase (26). Zamencik et al. (27) have demonstrated the presence of a peptidase, capable of hydrolyzing several synthetic peptides, in the lymph and serum of dogs. This peptidase activity increases in both fluids to a significant extent after injury. The possibility exists that this increase in proteolytic enzyme might result in an increased amount of protein fragments in the blood. However, it has been reported that experimental burns do not give rise to polypeptidemia, as measured by the difference between the substances precipitable by tungstic acid and by trichloroacetic acid (28). Other reports have indicated that there is some rise in the peptide level of the blood after severe injury (29, 30).

Amino Acids

Man et al. (31) have reported finding a sharp depression in the plasma amino acid level after surgical trauma. This decreased amino acid level was prolonged until healing was largely completed. Data from other reports (32, 33) have indicated that there is a small decrease in the level of some amino acids, and especially some of the “essential” ones, in the plasma after wounding. In these studies (32, 33) the differences that were indi-
cated seemed to be more related to the nutritional state than to the trauma. Several papers indicate that there is an increase in the plasma amino acid levels in several species after burns (9, 34-37). Although the level of several amino acids increased in the plasma after burns, there were particularly large increases in the histidine, tyrosine, and phenylalanine levels. However, the largest part of the increased plasma amino acid fraction was due to the presence of a compound which behaves, chromatographically, like taurine, a metabolite of cystine. Increases in the plasma urea (36, 109) and uric acid (12, 109) have also been observed following trauma.

NITROGEN METABOLISM

The most striking change of protein metabolism which results from injuries is the appearance of a negative nitrogen balance, frequently even in young or growing animals. Practically every paper considering metabolic aspects of the healing of wounds has demonstrated or noted the posttraumatic negative nitrogen balance. The increase in excreted nitrogen seems to be due primarily to an increase in the excretion of urea, although somewhat larger than normal amounts of amino acids, creatine, ammonium ion, and uric acid may also appear in the urine (9, 39-43). It has also been reported that there is a large increase in the "undetermined" nitrogen of the urine after burns (37, 42).

A chromatographic analysis has been made of the amino acids excreted in the urine after burns and surgical trauma (41). The rise in the amounts of amino acids excreted appears to be roughly correlated with the severity of the trauma. Although some increased excretion of all the amino acids was noted, the greatest increase appeared to be in the high-molecular-weight "essential" amino acids. Care must be taken in interpreting many of the results reported in papers such as that noted above, since the pattern of urine constituents may be markedly altered by unrelated stimuli. For example, barbiturates will cause a decrease in urea excretion with a concomitant increase in amino acid excretion in both normal and wounded animals (48).

Protein Metabolism

The metabolism of proteins gives rise to excretory products containing sulfur, as well as nitrogenous ones. The excretion of these sulfur-contain-

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8 In this discussion, the use of the word "cystine" implies both cystine and cysteine, unless otherwise specifically noted. This usage may be considered permissible at present, since the reduced and oxidized forms of this amino acid are interconvertible in vivo, and since the functional form of the amino acid in the processes under discussion is still undetermined.
ing compounds might be expected, and have been shown, to increase during the negative nitrogen balance observed after experimental wounds have been inflicted (43–46, 50). However, the amount of sulfur excreted was noted to be less than the nitrogen/sulfur ratio in the body tissues would lead one to expect, indicating a net retention of sulfur.

**Effect of Protein on Wound Healing**

Many experiments have been reported in which attempts have been made, by various methods, to decrease the negative nitrogen balance and also to increase the rate of healing of the trauma studied. The expected protein-sparing effect of a high-carbohydrate diet does not seem to be effective in decreasing the excretion of nitrogen or altering the rate of healing of wounds. However, the feeding of a high-protein diet not only increased the rate of healing of wounds over that displayed when a low protein diet was fed, but also resulted in a closer approach to nitrogen balance (2, 44–46, 54–63, 71, 74). Results which might be considered typical of this kind of experiment are shown in Fig. 1. It is most important to realize that the healing of wounds will take place when a very low protein diet is fed, and even in fasting animals (3, 44, 47, 60–63, 70, 71). This is also illustrated in the data presented in Fig. 1, where there is a large increase in tensile strength (the measure of healing) of the wounds even in the animals receiving the low-protein diet. In order

**Fig. 1.** The rate of healing of experimental wounds in rats fed isocaloric amounts of a high- and low-casein diet. The K values represent the slopes of the lines and are a numerical evaluation of the rate of healing. Data from Williamson, McCarthy, and Fromm (44).
to inhibit wound healing completely an appreciable period of starvation seems necessary so that a large part of the body weight is lost prior to the infliction of the wound (65). With this information in mind, one is tempted to suggest that the materials used for the regeneration of wound-tissue protein comes primarily, if not exclusively, from the tissue protein.

The mere feeding of a high-protein diet, without taking into account the constituents of the protein, is not sufficient to promote an increased rate of wound healing. Thus, feeding wounded animals with a diet which was high in protein (gelatin) did not significantly alter the rate of healing or the nitrogen balance from that observed when a low-casein diet was fed (44, 45). It then seems obvious that it is not the excess protein per se which is involved in increasing the rate of wound healing, but rather that the extra protein in the high-protein diet must be providing a larger amount of one or more amino acids which are in short supply, or which are required to a larger extent for the healing processes. As a corollary, it is reasonable to suppose that the requirements of the healing processes for relatively excessive amounts of one or more amino acids, supplied by the tissue proteins, leaves large quantities of the remaining amino acids in such a balance that they cannot be used for protein synthesis (68), but can only be metabolized for energy purposes. The residual nonutilizable amino acid nitrogen is then excreted and results in the negative nitrogen balance found after injury.

Effect of "Essential" Amino Acids

The effect of several "essential" amino acids on the healing of wounds and the excretion of nitrogen during this time has been studied. Lysine, tryptophane, and valine were found to have no significant effect on the rate of healing of experimental wounds when used to supplement a low- or high-protein diet (55). Similarly, a histidine supplement to a low-protein diet has no effect on the rate of healing or on the negative nitrogen balance (52). The administration of methionine to wounded or burned animals has been shown to accelerate the rate of healing and to decrease the excretion of nitrogen (6, 46, 49, 50, 66, 67, 73). The administration of cystine, but not cysteine, produces the same effect as that shown by methionine (47, 66, 67) (Fig. 2). The mechanism which makes cystine utilizable, but cysteine of no value, for the healing processes is not known.

The over-all reaction for the conversion of methionine to cystine in vivo has been demonstrated to be irreversible (75–77). It then follows
Fig. 24. The rate of healing of experimental wounds in rats fed a 6% casein diet supplemented with equimolar amounts of different amino acids. (A) Alanine supplemented to the diet; (B) methionine supplement; (C) cystine supplement. Data from Williamson and Fromm (47). 28. The same as the above except that the basal diet contained no protein (47).

that cystine is the principal amino acid in short supply during the period of wound healing; the negative nitrogen balance may be considered to be the result of an attempt to meet the requirement for an extra supply of cystine. The ability of methionine to increase the rate of healing and to decrease nitrogen excretion in wounded animals would seem to be due to the fact that it can be readily converted to cystine by the injured organism. Of course, this does not infer that the other amino acids are not required for the processes involved in the healing of wounds.6

The retention of sulfur by the wounded animal, the excess requirement for cystine, and its ability to increase the rate of healing all point to the possibility that the cystine is utilized primarily in the synthesis of protein. Since the injured animal displays a marked protein catabolism and the principal anabolic activity occurs in the wound area, it would be expected that the cystine was being used for the production of wound proteins. A further discussion of the proteins in regenerating wound tissue will be considered in the section “Wound Proteins.”

6 It has recently been reported that cystine is required for the extended growth of fibroblasts in vitro (110). Fibroblasts may survive for several days without any of the sulfur amino acids being present in the growth medium. However, the presence of cystine alone, but not methionine alone, was shown to extend the growth period to more than a month. The best growth was observed when both amino acids were present. These results are a further confirmation of the importance of cystine during the regeneration of tissue and growth.
Metabolic Reactions

The general metabolic reactions of injured rats have been studied with the use of N\(^{15}\)-labeled glycine (78) and C\(^{14}\)-labeled glycine (79). In both of these series of experiments, it was shown that the labeled compounds never reached as high a level in the visceral organs of the injured animals as in the normal unwounded controls. This effect may be due either to a reduced uptake or to a more rapid turnover of the labeled atom. The latter situation would seem to be indicated from the fact that the injured animals respired more C\(^{14}\)O\(_2\) than did the controls. Experiments using methionine and cystine labeled with S\(^{35}\) indicate that the rate of sulfur metabolism is increased during the healing of wounds (49–51), the turnover of the S\(^{35}\) being more rapid in injured animals as compared with the normal controls. Some of the results of these experiments seem to suggest that methionine from the liver supplies a large part of the cystine required by the regenerating wound tissue.

The metabolic activity in the liver after injuries has also received some attention. It has been shown that the deamination of alanine at the height of nitrogen excretion after burns is quite appreciably lower than in control animals (78). This may account in part for the increased excretion of amino acids observed after wounding (41, 109). It has also been reported that the liver lipids drop markedly from normal levels for several days after injury (81). This reduction in liver lipids is reported to be, at least partially, reversed by the administration of methionine or cystine. It might be postulated that the decrease in liver lipids and the increased level of plasma lipoproteins (12, 18) are merely two aspects of the same phenomenon. In spite of the reported changes in the liver constituents and metabolism, it has been shown that the oxygen consumption of liver slices from burned animals is much the same as that found in normal animals (82).

Since several hormones are known to affect the metabolism of proteins, a short discussion of the effect of some of these hormones on wound healing would be in order. It has been demonstrated that when thyroidectomized animals are wounded they excrete considerably less nitrogen than do wounded nontyroidectomized controls, while no appreciable change in the rate of healing is observed. On the other hand, the administration of thyroxin or pituitary thyrotrophic hormone depresses the rate of healing markedly, but only slightly increases the excretion of nitrogen (83–89). Although some of the androgenic steroids appear to depress the excretion of nitrogen to some extent after wounding, they also inhibit the rate of healing (84, 85, 89–92). Pituitary growth hormone appears to
inhibit the rate of healing when administered at relatively high dosage levels, but increases the healing rate when smaller amounts are given (53, 89). Finally, several of the steroids from the adrenal cortex have been shown to have a strongly inhibitory effect on the healing processes, but appear to have little, if any, effect on the posttraumatic excretion of nitrogen (93–99).

WOUND PROTEINS

The work on the structures and the transformations which occur in wound tissue has been approached, for the most part, from the histologic point of view. Still, there has been some information accumulated about the chemical characteristics of the substances being synthesized in the regenerating wound tissue. An extensive study has been reported by Orekhovitch (100) on two of the important proteins which appear in regenerating wound tissue. One of the early proteins to appear in the wound tissue is a procollagen “which has an intrinsic role in the regeneration processes.” The procollagen serves as a precursor and substrate for the formation of collagen fibers, found in the healing wound. Both the procollagen and collagen appear to have similar properties to collagens and procollagens isolated from sources other than regenerating wound tissue. An amino acid analysis of the purified proteins was also reported.

Fig. 3. The concentration of nitrogen, methionine, and cysteine and cystine in the regenerating wound tissue of rats plotted against days after wounding. Methionine values are represented by open circles; nitrogen by half-open circles; cysteine and cystine by solid circles. All the values are shown in terms of milligrams per Gram of wet tissue, except for the nitrogen, which is in terms of 0.1 of actual value. Data from Williamson and Fromm (51).
The wound collagen and procollagen consist largely of the prolines and the simpler amino acids; they do not contain all of the common amino acids; they contain only a small percentage of methionine and little, if any, cystine.

Cystine-Methionine Content of Wound Tissue

Some of the proteins which are synthesized in the wound tissue appear to contain appreciably more cystine and methionine than does either the normal skin tissue, the wound collagen, or procollagen. The regenerating wound tissue, at the maximum level, has been shown to contain 170 mg. of methionine and 220 mg. of cystine per Gm. of nitrogen as compared with 115 mg. of methionine and 75 mg. of cystine per Gm. of nitrogen in normal skin (51). From the rate of deposition of the sulfur-containing amino acids and nitrogen, shown in Fig. 3, it can be seen that there are two distinct types of sulfur-rich proteins being produced in the regenerating wound tissue. During the early stages of healing, the wound tissue is accumulating a larger proportion of proteins that are relatively high in methionine and low in cystine. As the healing progresses, proteins having a higher percentage of cystine than methionine are synthesized and deposited in the healing wound tissue.

![Graph](image)

**Fig. 4.** Tensile strength of healing wounds in rats fed a protein-free diet plotted against the cystine and cysteine content of the regenerating wound tissue. Open circles are data from rats given a methionine supplement in the diet. Solid circles represent data from rats given an equimolar supplement of alanine in the diet. Data from Williamson and Fromm (46).
Since the strength of wound tissue appears to be due to its content of collagen fibers, the methods for measuring the rate of healing of wounds which employ a tensile-strength or wound-rupture technic are actually measuring the rate of deposition of collagen fibers in the wound area. In spite of the lack of cystine in collagen, there appears to be an excellent correlation between the amount of cystine deposited in the proteins of the wound tissue and the tensile strength of the wound, as shown in Fig. 4. This may be explained by assuming that the cystine-rich proteins are being produced in the wound tissue at approximately the same rate as is the collagen.

**Sulfur and Sulfate Uptakes**

The uptake of sulfur in the form of amino acids must not be confused with the uptake of sulfate ion by the healing wound, which has also been reported (101, 102, 111). While the sulfur amino acids are used for the synthesis of protein, the sulfate ion is incorporated into the mucopolysaccharides of the ground substance associated with the collagen fibers of the wound tissue (103). However, the sulfate ion produced from the metabolism of the sulfur-containing amino acids may be used for the latter function.

**Enzymatic Proteins**

Proteins with enzymatic properties have also been identified in the regenerating wound tissue. A proteolytic enzyme has been shown to be present in wound tissue by many workers (40, 104, 105). Whether this proteolytic enzyme is the same as the one detected in the plasma (27), and whether it originates in the body tissues and migrates to the wound tissue, or vice versa, is not known. At present, we can only guess at the function of this enzyme in the economy of the wound tissue. The healing wound tissue has also been shown to have a high level of phosphatase activity (106, 107). Since collagen fibers have been shown to bind phosphatases very strongly in vitro (108), it is possible that the origin of this enzyme in the wound tissue is due to its accumulation from body tissue sources. Again, in this case, the origin and function of this enzyme can only be surmised.

**CONCLUSIONS**

The foregoing is illustrative of the available information on the alterations in the biochemical processes which occur during the healing of wounds. Many of the studies mentioned appear to make some aspects
of the phenomena involved in the healing of wounds more understandable. Yet, these results are too isolated to form a clear and unified picture of the healing processes and the mechanisms which control them. It will be necessary to discover the mechanisms which bring about the changes in metabolism before a real understanding of the processes of wound healing can be obtained. Obviously, much more data are required before these mechanisms can become apparent.

REFERENCES