Changes in the Serum Protein Levels During Wound Tissue Regeneration

Lilita DiLallo, Harold B. Haley, and Martin B. Williamson

The effect of wounding and formation of regenerating wound tissue on the level of the serum proteins was studied in rats on a protein-free diet. It was found that the level of the globulin component having the third greatest electrophoretic mobility, probably part of the $\alpha_2$-globulin fraction, rose about 60% within 3 days after wounding and did not begin to return to normal until about 10 days later. Concomitantly, the level of the globulin with least mobility, presumably the $\gamma$-globulin component, decreased about 35% and also began to return to normal within 2 weeks after wounding. Varying the rate of healing, by the addition of protein or methionine to the diet, had no effect on the changes in the serum globulin levels. The newly formed $\alpha_2$-globulins were shown to be distinct from fetuin.

The relative amounts of the protein components in serum have been reported to change considerably during wound tissue regeneration. The most characteristic of these changes appears to be a decreased concentration of albumin and an increase in the $\alpha$-globulins (1-11). The relation of these new levels in the serum proteins to wounding or tissue regeneration is obscure. Hormonal factors have been suggested to be involved in altering the level of serum proteins after injury (4, 22). Some evidence in support of the idea that the $\alpha_2$-globulins act as detoxifying agents for the metabolic products of the wound debris has been presented (24).

Regeneration of wound tissue is accompanied by profound changes in the metabolism of proteins and amino acids. On a protein-deficient diet the rate of tissue regeneration is slower than when an adequate diet is fed (12-15). Conversely, the rate of wound tissue regeneration

From the Departments of Biochemistry and Surgery, Graduate School and Stritch School of Medicine, Loyola University, Chicago 12, Illinois.

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can be increased in protein-depleted animals by feeding them a high-protein diet (16, 17). If, instead of protein, the sulfur amino acids, methionine or cystine, are supplemented in the protein-deficient diet, the rate of tissue regeneration is similarly increased (17–20). It has been shown that proteins containing relatively large amounts of sulfur amino acids accumulate in the regenerating wound tissue (12); the amount of these amino acids in the wound tissue increases as the regeneration proceeds (21). Thus, the increased amounts of dietary sulfur amino acids appear to be required for adequate wound tissue regeneration to take place; the availability of these amino acids appears to be a limiting factor in determining the rate of healing.

Since the level of the dietary sulfur amino acids is of such basic importance in regulating the rate of regeneration of wound tissue, the question arose as to whether the changes in the serum globulin levels, observed after wounding, are affected also by the availability of these amino acids. If the serum proteins are related in some way to the process of regeneration, changing the rate of healing should be reflected in some change in serum protein level. The present report considers the effects of dietary factors on serum protein composition in wounded animals in which the rate of tissue regeneration has been decreased by protein depletion.

**Experimental**

The following experiments were carried out on female albino rats weighing 220 ± 20 gm. at the start of the experiment. The animals were kept on a protein-free diet (12) from 3 days prior to wounding until the termination of the experiments. The animals were individually housed after wounding to prevent disturbance of the regenerating tissue. Each animal was offered 8 gm. of the diet daily; this amount of diet was found to be completely consumed in the 24 hr. before the next feeding time. Water was permitted ad libitum. The animals were wounded while under Nembutal anesthesia. A standard wound was made by excising a circular piece of skin and subcutaneous tissue, 4 cm. in diameter, on the back of the neck down to the loose fascia (10). Control animals were subjected to the identical treatment except for the wounding.

Blood samples were obtained daily from the tip of the tail. After application of a small amount of xylene, the tail was wiped dry and the skin at the tip end cut off. Approximately 0.1 ml. blood was collected by capillarity in one end of a melting-point tube which had a volume of
about 0.2 ml. The opposite end of the tube was sealed off by heat. The
blood was permitted to clot for 15 min. at room temperature and then
centrifuged to separate the serum. Samples were discarded if any
perceptible hemolysis was observed. On successive days, blood was
obtained by removing the scab from the small wound on the tip of the
tail. Total protein concentration in the serum samples was determined
by the method of Lowry et al. (25); a pooled sample of lyophilized and
reconstituted rat serum was used as the standard. It was noted that
the protein concentration in the serum obtained from the tail was 25–
30% higher than in serum collected by heart puncture in the same rat
at the same time.

Electrophoretic separation of the serum protein fractions was car-
rried out by the horizontal technic in veronal buffer at pH 8.6, ionic
strength 0.05 (26). Cellulose acetate strips were used as the support-
ing medium (27, 28). The serum was diluted with three parts of veron-
al buffer, resulting in a solution containing approximately 1.8–2.5
gm./100 ml. of protein. About 40 μg. of protein in a volume of 2 μl. of
solution were applied in a thin streak to the cellulose acetate strip. The
electrophoresis was carried out at 4°, using a potential gradient of 20
v/cm. of cellulose acetate strip for 135 min. It was found that by this
procedure the proteins in serum samples could be separated into 7 well-
defined fractions.

Directly after the electrophoretic separation, the serum proteins
on the electrophoretograms were stained by immersion in a solution of
0.2% Azocarmine B in 5% acetic acid for 30 min. (29). Excess stain
was removed by rinsing the strips in 5% acetic acid for another 30 min.

No attempt has been made to determine the relationship of the
amount of dye taken up by each fraction to the actual amount of pro-
tein present in each fraction. The purpose of these experiments was
not to compare the different protein components in the same serum,
but rather to compare corresponding fractions of sera from wounded
animals and from the unwounded controls. Therefore, it was not con-
sidered necessary to determine the absolute amount of dye which com-
bined with the protein in each fraction.

The intensity of color of the protein fractions on the strips was
measured at 580 mμ by means of a recording densitometer described
by McDonald (30). The wave length of the incident light of the densi-
tometer was adjusted by means of a grating monochromator; the
transmitted light was amplified and traced on a strip chart recorder
through a logarithmic converter. From the areas under the curves of the tracings obtained, the protein concentration was determined. The amount of protein in each fraction was calculated from the equation:

\[ P_a = \frac{(A_a) (P_0)}{A_t} \]

where \( A_a \) is the area under the curve for fraction \( a \); \( A_t \), the sum of the areas under the curves for all the fractions; \( P_0 \), grams of protein per 100 ml of serum as determined by analysis; and \( P_a \), grams of protein in fraction.

**Results and Discussion**

The electrophoretic pattern of a representative serum sample from a normal animal, as obtained from the strip chart recorder, is shown in Fig. 1. In contradistinction to the five components usually obtained from electrophoresis of serum protein on filter paper, the technique used here separates the serum proteins into seven components. The two extra components probably arise from a further separation of the \( \alpha \)- and \( \beta \)-globulins.

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**Fig. 1.** Strip chart recorder pattern of electrophoretically separated fractions of serum proteins on cellulose acetate strips stained with Azocarmine B and color intensity measured in a densitometer at 580 mµ.

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**Fig. 2.** Concentration of albumin in sera (gm./100 ml.) of normal and wounded rats, plotted against days after wounding.
In Fig. 2 are shown the changes in the albumin concentration in the serum from wounded and unwounded rats. It should be noted that the level of albumin in normal rats decreases with time; this is due to the fact that the animals are being maintained on a protein-free diet. The levels of serum albumin in the wounded rats decrease rapidly and significantly beyond the decrease observed in the normal animals. These data are in agreement with previously recorded results (2–7).

The third component of the globulin fractions, shown in Fig. 1, increases significantly directly after wounding and only begins to return toward normal after about 10 days. These changes are plotted in Fig. 3. It seems highly probable that this fraction is part of the $\alpha_2$-globulins which have been reported to increase after injury (2–9).

Simultaneously with the increase in the globulins in Fraction 3, the level of the globulin component with the lowest electrophoretic mobility decreases significantly and does not begin to return to normal until about 10 days after wounding. The effect of wounding and regeneration on the level of globulin Fraction 6 is shown in Fig. 4. On the basis of mobility, this fraction appears to correspond to the $\gamma$-globulins. Other reports concerning $\gamma$-globulin levels following injury are rather
contradictory and inconclusive (5, 6, 8, 9). Probst et al. (9) and Schumacher (24) found no change, or only a slight increase in γ-globulin concentration in the serum following surgical wounds. A questionably significant decrease in γ-globulin levels, attributed to shifts in body fluids, has been reported after burn injury in humans (31, 32) and in rats (3).

Since the animals in the experiment reported here had been kept on a protein-free diet, it would be expected that the rate of tissue regeneration in these animals would be slower than if they had been adequately fed (12–15). To determine whether the changes in the levels of the serum proteins in wounded rats are influenced by these factors, a comparison was made of the serum protein fractions obtained from wounded rats kept on a protein-free diet and those fed a diet containing 20% casein. It can be seen in Fig. 5 that neither the level of protein in the diet nor the resultant difference in the rate of healing had any effect on the changes in the level of globulin in Fraction 3 and Fraction 6 on the tenth day after wounding; neither were significant differences observed on the sixth and eighth days after wounding. The changes in the albumin level may be attributed to the difference in the protein intake.

Since the effect of the sulfur amino acids on the rate of healing is masked by a high-protein diet (14), it was undertaken to study the effect of the sulfur amino acid methionine on the changes in the levels of globulin fractions when a protein-free diet was fed. Control animals received the protein-free diet supplemented with an equal amount of nitrogen in the form of glutamic acid. As shown in Fig. 6 and 7, there appears to be no significant difference in the levels of these globulin fractions after wounding. From these results it is evident that neither the increase in Fraction 3 nor the decrease in Fraction 6, which

**Fig. 5.** Effect of level of protein in diet on relative concentration of serum proteins on tenth day after wounding.
accompanies injury, is affected by dietary conditions which are able to influence the rate of tissue regeneration. It follows, then, that the changes in serum protein composition which accompany tissue injury are unrelated to processes which control the rate of healing.

![Graph](image1.png)

**Fig. 6.** Concentration of globulin Fraction 3 in sera of wounded rats fed a protein-free diet. Open circles indicate 0.002 moles methionine supplemented per 100 gm. diet; solid circles, 0.002 moles glutamic acid supplemented per 100 gm. diet.

![Graph](image2.png)

**Fig. 7.** Concentration of globulin Fraction 6 in sera of wounded rats fed a protein-free diet. Open circles indicate 0.002 moles methionine supplemented per 100 gm. diet; solid circles, 0.002 moles glutamic acid supplemented per 100 gm. diet.

It has been reported that fetal serum contains an α-globulin component (fetuin) which appears to have growth-promoting properties as tested on the proliferation of cells in tissue culture (33). Since the healing of wounds is a growth phenomenon, the possibility that the Fraction 3 globulin might be related to fetuin was investigated. A comparison was made between the electrophoretic patterns of serum from adult wounded and normal fetal rats. It was found that the serum from fetal rats contained a relatively high concentration of a protein with an electrophoretic mobility similar to α-globulins. However, this fraction does not correspond in mobility to the Fraction 3 in the serum from wounded rats when fractionated on the cellulose acetate strip. A further difference between the fetuin and the α₂-globulins was observed: it was found that the Fraction 3 globulin is precipitable with 0.5 saturated ammonium sulfate, while the fetuin from the serum of fetal rats remained in the supernatant fluid with the albumin.
References