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Received July 7, 1952. P.S.E.B.M., 1952, v80.

Effect of Cystine and Methionine on Healing of Experimental Wounds.* (19712)

MARTIN B. WILLIAMSON AND HERBERT J. FROMM.
From the Department of Biochemistry, School of Medicine and Graduate School, Loyola University, Chicago.

In previous reports(1,2), the more rapid rate of healing of experimental wounds in animals fed a high protein diet as compared to those on a lower protein diet was shown to be due to the greater intake and retention of protein sulfur. It was also shown that methionine could serve as the source of protein sulfur. This explained earlier work which had indicated that methionine increased the rate of healing(3-5). Other essential amino acids (6,7) were found to have no effect on the *healing index,* a numerical measure of a function which is proportional to the rate of healing.

Methionine may have 2 possible roles, involving its sulfur atom, which might affect the *healing index.* First, it might be required directly in the healing processes for such reactions as protein synthesis. Secondly, methionine might serve as a precursor for some other sulfur-containing compound required during healing. Although these alternatives need not necessarily be mutually exclusive, it is not unreasonable to presume that one function will be more important than the other.

In the present paper, it is shown that the *healing index* can be affected by cystine to the same extent as by an equivalent amount of methionine, on the basis of sulfur.

Experimental. The experiments to be described were carried out in a similar manner to those previously reported(1,2). In each experiment, 3 groups of 24 female albino rats (200 ± 20 g) were maintained on a basal diet for 5 days prior to wounding. The basal diet consisted of 6 g casein, 10 g lard, 2 g corn oil, 5 g salt mixture(8), 77 g sucrose, 1500 I.U. vit. A,† 210 I.U. vit. D,† 1 mg thiamine HCl, 1 mg riboflavin, 1 mg pyridoxine HCl, 15 mg nicotinic acid amide, 4 mg calcium pantothenate, 0.5 mg 2-methyl naphthoquinone, 5 mg inositol, and 25 mg choline chloride. This diet would permit only a small amount of protein accretion in normal unwounded animals (as measured by nitrogen excretion and increase in body weight). On the day of wounding, the animals were transferred to the experimental diets. All the animals were given the same weighed amount of diet daily, in quantities which would be completely consumed. Distilled water was permitted *ad libitum.*

Standard experimental wounds were made on the back of the neck of the rats as previously described(1). At approximately weekly intervals, 1/3 of the animals in each group were sacrificed and the tensile strength of a number of 0.5 cm sections of the healing wound were measured(1,9). The relationship of tensile strength to time results in a curve which may be considered to be essentially a straight line. This line can be represented by the equation $T = kt + C$, where T is the tensile strength in grams, and t, the time in days. C is a constant. The slope of this line (K) is the *healing index,* and may be computed from the equation:

$$K = \frac{T_2 - T_1}{t_2 - t_1}$$

where T_1 is the tensile strength at time t_1, and T_2 is the tensile strength at time t_2. K

* This work was done under contract with the U. S. Navy, Office of Naval Research.

† From oleum percomorphum.

TABLE I. Effect of Experimental Wounds on the Sulfur and Nitrogen Balances.
Exp. I.

Group No.	Casein in diet, %	Amino acid supplement	Nitrogen per 100 g diet, mg	Sulfur per 100 g diet, mg	Nitrogen: Avg daily intake, mg	Nitrogen: Avg daily retention, mg	Sulfur: Avg daily intake, mg	Sulfur: Avg daily retention, mg	"Excess sulfur," mg	*Healing index*
I	6	Alanine	910	48	64.8	6.9	3.4	1.53	1.07	40
II	6	Methionine	910	248	64.8	22.1	17.7	4.15	2.68	50
III	6	Cystine	910	248	64.8	22.7	17.7	4.22	2.64	51

has the dimension of a rate term which describes a function of the rate of healing.

Urine samples were collected daily before and after the time of wounding. The urine was stored under toluene at 5°C until analyses were run. The urine was analyzed for total nitrogen (microkjeldahl) and sulfur(10,11).

Results and discussion. The nitrogen and sulfur sources of the diet fed the wounded animals used in Exp. 1 are described in Table I. The *healing indices* for these animals were calculated from the tensile strength data plotted in Fig. 1. It can be seen that the rates of healing in the animals receiving the methionine and cystine supplements ($K = 50$ and 51) are significantly greater than that of the control group ($K = 40$). It should be noted particularly that equivalent amounts of cystine and methionine (on the basis of sulfur) have the same effect on the *healing index*. A repetition of this experiment gave identical results.

A correlation between the sulfur retention and the *healing index* was observed here, as in previously reported work(1,2). It would be expected that the retention of sulfur should be proportional to the retention of nitrogen, in a ratio similar to that found in the animal. In the rat, the nitrogen:sulfur ratio is approximately 15:1. After wounding, there appears to be a greater retention of sulfur than might be expected from the amount of nitrogen which is retained. This "excess sulfur" also appears to be correlated with the *healing index*. The data supporting this correlation are shown in Table I.

Whether the results noted above were due to the direct action of the cystine supplement, or to the effect of the cystine in sparing the methionine available from the casein in the diet, remained to be determined. Therefore, an experiment similar to the previous one was carried out, except that the casein was omitted from the diet fed the wounded animals. The control group of animals received a diet containing no sulfur amino acids and 44 mg of amino acid nitrogen per 100 g diet, in the form of alanine. The cystine and methionine supplemented diets contained 100 mg of amino acid sulfur and 44 mg of nitrogen per 100 g of diet. The curves of tensile strength against time obtained in this experiment are plotted in Fig. 2.

Here again, the effect of cystine and methionine on the *healing index* can be seen to be essentially the same ($K = 36$ and 34) and significantly greater than that found in the

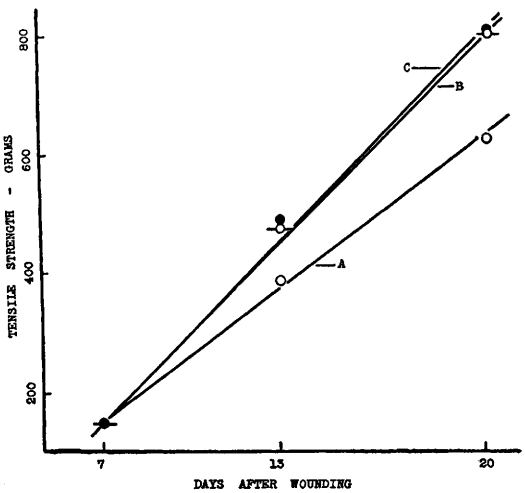

FIG. 1. Tensile strength of healing wounds in rats on a 6% casein diet plotted against time. Curve A (Group I), alanine supplement, *healing index (K)* = 40; Curve B (Group II), methionine supplement, *(K)* = 50; Curve C (Group III), cystine supplement, *(K)* = 51. The significance between mean values of tensile strength for Groups I and II is "p" = <.01.

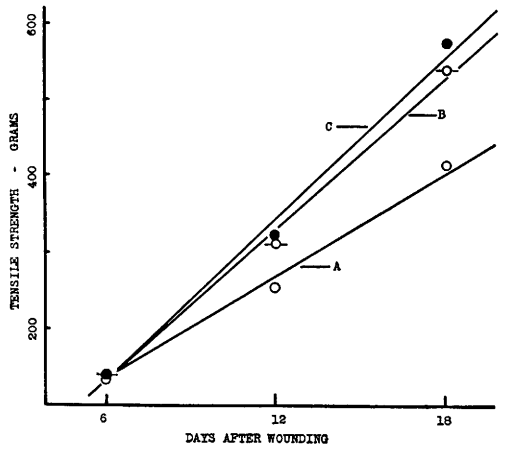

FIG. 2. Tensile strength of healing wounds in rats on a non-protein diet plotted against time. Curve A (Group I), alanine supplement, *healing index (K)* = 24; Curve B (Group II), methionine supplement, $K = 34$; Curve C (Group III), cystine supplement, $K = 36$. Significance between mean values of tensile strength for Groups I and II is "p" = <.01.

controls ($K = 24$). Since the conversion of methionine to cystine is irreversible *in vivo* (12,13), it must be concluded that the methionine in the diet is first converted to cystine before it becomes available for the healing processes. It then appears that cystine is the limiting factor affecting the *healing index,* and that methionine serves primarily as a source of cystine sulfur. Of course, cystine, as such, may not be required by the healing wound. It may be that cystine is itself merely a precursor of the sulfur containing substance utilized during healing. The correlations between the *healing indices,* the sulfur balances and the "excess sulfur" values for this experiment are shown in Table II.

Further work has indicated that methionine, *per se,* is required to some extent during wound healing, over and above that which may be converted to cystine. When rats were fed a 5% casein diet, supplemented with 100 mg of ethionine sulfur and 100 mg of either cystine or methionine sulfur per 100 g diet, a lower *healing index* was observed than in the control animals, who received no sulfur amino acid supplement. A comparison of the tensile strength data for this experiment is shown in Fig. 3. These data may be interpreted to mean that the ethionine is interfering with the utilization of both the cystine and the methionine.

Ethionine is known to block the conversion of methionine to cystine as well as the incorporation of methionine into protein(14,15). The latter effect results in a decreased rate of protein synthesis. In Group II (methionine supplement), the low *healing index* may be considered to be due to the lack of cystine resulting from the interference with methionine conversion. However, in spite of the relatively large amounts of cystine available to the animals in Group III, a low *healing index* was still observed. In this case, it seems probable that the ethionine interfered with the utilization of methionine for purposes other than cystine formation, so that the cystine requirement was no longer the limiting factor in the healing process. It must then be concluded that methionine is also required for wound healing. It is not unlikely that the methionine requirement during wound healing is needed primarily for protein synthesis, whereas, the cystine required may be used, to some extent, for reactions other than protein synthesis.

In the experiment where the diet fed to the wounded rats contained no protein, the methionine required for healing by the control animals and by those receiving the cystine supplement must have originated in the tissue protein. It would then be reasonable to think that the methionine requirement must be

TABLE II. Effect of Experimental Wounds on Nitrogen and Sulfur Balances. Exp. II.

Group No.	Nitrogen		Sulfur		Avg "excess sulfur," mg	*Healing index*
	Avg daily intake, mg	Avg daily retention, mg	Avg daily intake, mg	Avg daily retention, mg		
I	3.1	−40.1	0	−2.06	.60	24
II	3.1	−35.9	7	1	1.39	34
III	3.1	−35.6	7	1.04	1.33	36

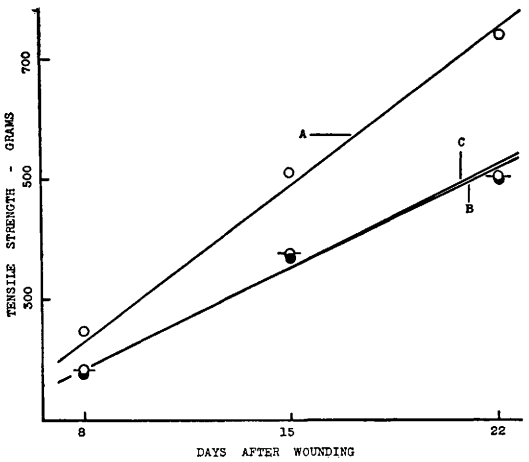

FIG. 3. Tensile strength of healing wounds in rats on a 5% casein diet plotted against time. Curve A (Group I), alanine supplement, *healing index (K)* = 31; Curve B (Group II), ethionine and methionine supplement, $K = 21$; Curve C (Group III), ethionine and cystine supplement, $K = 21$. Significance between mean values of tensile strength for Groups I and II is "p" = .01.

relatively small as compared to the cystine requirement.

Summary. The effect of methionine and cystine on the *healing index* of standard experimental wounds in rats was determined. Since both amino acids have the same effect, per equivalent of sulfur, it is concluded that methionine is converted to cystine before being used in the healing process. When the utilization of methionine is blocked by ethionine, cystine is ineffective, indicating that some methionine, *per se,* is required for the healing of wounds. There appears to be a correlation between the *healing index* and the retention of amino acid sulfur in excess of that expected on the basis of nitrogen retention.

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Received July 7, 1952. P.S.E.B.M., 1952, v80.

Effects of Auxotrophic Mutations on the Adaptation to Inositol Degradation in *Aerobacter Aerogenes.** (19713)

DAIZO USHIBA† AND BORIS MAGASANIK. (Introduced by J. Howard Mueller)

From the Department of Bacteriology and Immunology, Harvard Medical School, Boston, Mass.

Enzymatic adaptation and cell division in microorganisms are closely allied. Both processes are inhibited by the same agents, and in many instances only dividing cells are capable of adaptation. When adaptation can occur in the absence of a source of exogenous nitrogen, lower levels of adaptive enzymes are attained than in media capable of supporting growth(1). It has been postulated that in resting cells an interconversion of enzymes is responsible for adaptation(1). On the other hand, very recently, evidence has been presented that resting yeast cells contain

* Supported by the William F. Milton Fund.

† Present address: Department of Bacteriology, Keio Medical School, Tokyo, Japan.