to the 2% protein diet did not elevate the value in 3 additional animals tested (Mean 26.8 ± 11.7). If the values for each diet are grouped and statistically tested(3), the P values for each depletion group compared to the control group are less than 0.01, indicating significant differences. Potter and Klug (17) have reported that succinate oxidation of liver was decreased on diets high in carbohydrate. Since the high carbohydrate diets contained only 6% casein as protein, there exists the possibility that the observed effect might be due in large part to this low protein diet.

Summary. Rats fed low protein or protein-free diets for periods up to 8 weeks exhibit (1) no change in skeletal muscle ATP-ase activity; (2) no change in liver cytochrome oxidase activity; (3) no change in liver arginase activity per gram of tissue, but a decrease in total arginase units; and (4) a marked decrease in the succinoxidase activity of liver.

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Relation of Protein Nutrition to the Healing of Experimental Wounds.* (18759)

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A number of reports have indicated that the level of protein intake has a marked effect on the rate of healing of wounds(1-8). There is, however, little work relating protein metabolism or the effect of amino acids to the healing of wounds(4-6,9,10). It has been shown that methionine will increase the rate of healing of experimental wounds(4), whereas tryptophane, valine and lysine have no significant effect on the rate of healing(10). Since greater nitrogen retention will occur when extra methionine is fed(11,12), it seems probable that the methionine, rather than other essential amino acids, is the limiting factor in the healing process. In fact, injured dogs will retain methionine sulfur and incorporate it into protein, even when a negative nitrogen balance exists(13). Beyond the fact that a negative nitrogen balance appears shortly after wounding(14,15), little seems to be known about protein metabolism during the healing of wounds.

Further study may help elucidate the role of protein metabolism in the healing process. The present paper attempts to relate nitrogen and sulfur metabolism to the rate of healing of experimental wounds.

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TABLE I. Composition of Diets.*

Group #	% casein	% gelatin	% supplemental methionine	g N/100 g diet
I	20	0	0	2.56
II	6	0	0	.76
III	6	11.3	0	2.55
IV	6	11.3	.147	2.57

* Sucrose content of diets was adjusted so that all diets provided same number of calories/100 g diet.

Experimental. In these experiments, four groups of 24 female albino rats were used. The initial average weight of the animals in each group ranged from 170 to 186 g. The animals in Group I were fed the following basal diet: sucrose, 63 g; casein, 20 g; lard, 10 g; corn oil, 2 g; salt mixture,† 5 g; thiamine HCl, 1 mg; riboflavin, 1 mg; pyridoxine HCl, 1 mg; calcium pantothenate, 4 mg; nicotinic acid amide, 15 mg; inositol, 5 mg; 2-methyl naphthoquinone, 0.5 mg; choline chloride, 25 mg; vitamin A,‡ 1500 IU; vitamin D,‡ 210 IU. The other groups of animals were fed diets, modified from the basal, as indicated in Table I. All the diets were isocaloric and contained the same amount of vitamin supplement as in the basal diet. Each animal was presented with 15 g of diet per day. The residual uneaten food was weighed to determine the daily food consumption. All animals were given distilled water, *ad libitum*. The animals were first accustomed to the synthetic diets for 4 days. Then they were wounded under nembutal and ether anesthesia. After clipping the hair over the area of the shoulder blades, a skin incision, 2 cm in length, was made with a sharp scalpel in the mid-dorsal region. The edges of the wound were opposed and held in place with Michel wound-clips (11 mm), which were left in place during the experiment. At approximately weekly intervals, 8 rats from each group were killed and the tensile strength of a 0.5 cm section of the healing wound was determined. The method of measuring the tensile strength of such wounds has been previously described(1).

† Hubbel, R. B., Mendel, L. B., and Wakeman, J. J., *J. Nutr.*, 1937, v14, 237.

‡ From Oleum percomorphum.

The rats were weighed and 24-hour urine samples were collected regularly, during the course of the experiment. The urine samples were stored under toluene at 5°C until the analyses were made. The urine was analyzed for total nitrogen (microkjeldahl), urea nitrogen(16), amino acid nitrogen(17), and total sulfur(18).

Results and discussion. Previous papers have indicated that a negative nitrogen balance appears directly after animals have been subjected to trauma(9,13-15). A sharp decrease in the amount of nitrogen retained, shortly after the animals were wounded, was observed in these experiments, although not all the animals went into negative nitrogen balance. The sulfur balance remained positive and essentially constant throughout the experiment, regardless of the changes in the nitrogen balance. By the tenth day after wounding, the daily nitrogen retention had returned to approximately the level observed before the wound was made. This is illustrated in Table II.

A marked decrease in urinary urea output (from 109 mg per day to 81 mg per day) with a concomitant increase in the amino acid nitrogen output (from 6.1 mg per day to 26.6 mg per day) was observed shortly after the

TABLE II. Nitrogen and Sulfur Retention Before and After Wounding.

Group #	Avg retention before wounding*		Retention on 2nd day after wounding*		Retention on 10th day after wounding†	
	N, mg	S, mg	N, mg	S, mg	N, mg	S, mg
I	92	11.3	44	11.2	85	10.1
II	9	2.8	−11	1.5	8	2.3
III	34	6	14	3.8	39	6.6
IV	52	9.2	15	11.1	42	13.4

* Computed from analyses obtained from 24 animals per group.

† Computed from analyses obtained from 16 animals per group.

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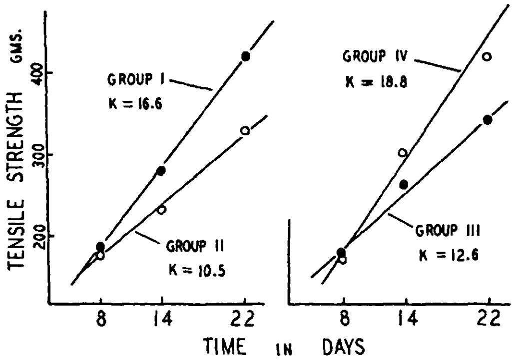

FIG. 1. Tensile strength plotted as function of time in days after wounding. The slope (K) is an index of the rate of healing.

wounds were made.§ The urea nitrogen and amino acid nitrogen returned to approximately the normal, prewounding levels within 5 days. This effect was found to be due to the anesthetic used and not to the wound.

In Fig. 1 is shown the rate of healing of the experimental wounds in terms of tensile strength and time. This can be expressed as, $T = K\ t$, where T is the tensile strength in grams per 0.5 cm section of healing epithelium, t, the time in days after wounding, and K, the resulting slope. The slope, K, can be considered to be a measure of the rate of healing of the wounds. It will be seen that the rate of healing of the wounds in the animals fed the 6 percent casein diet (Group II; K = 10.5) is markedly less than that of the animals on the 20% casein diet (Group I; K = 16.6). This is in agreement with previous work which has indicated that wounds in animals on a high protein diet heal more rapidly than those in animals on a low protein diet(1-4). From this, it would be expected that the rate of healing of the wounds in animals in Group III should be the same as those in Group I, since the protein nitrogen intake in both groups was the same. Instead, the slope (K = 12.6) indicates that the rate of healing is very much less than that found in Group I. This difference in healing rate is obviously due to the very low biological value of the gelatin, supplemented to the diet of the animals in Group III to make it contain the same amount of protein nitrogen as is found in the diet of Group I.

Although the proteins ingested, and the protein nitrogen intake, is essentially the same in the animals comprising Groups III and IV, the rate of healing of Group IV (K = 18.8) is very much greater than that for the animals in Group III. This difference must be attributed to the methionine added to the diet of the rats in Group IV. It can then be concluded that the methionine increases the efficiency of utilization of dietary protein or tissue protein, or both. Since the methionine fed is probably available to the tissues for protein synthesis as cysteine and cystine, as well as methionine(19,20), all the sources of dietary sulfur might be considered together as "protein sulfur."

There appears to be no correlation between the protein nitrogen intake, or retention, and the tensile strength or rate of healing, when all 4 groups of animals are considered. The data in Table III, however, indicate a correlation between the amount of "protein sulfur" retained during the course of the experiment and the rate of healing of the wounds. It seems evident from these results that previous reports(1-8) on the effect of the level of protein in the diet on the rate of healing were indicative of the effect of the level of "protein sulfur" intake (and indirectly, the sulfur retention), rather than the effect of the protein itself. This is apparent from the fact that previous reports dealt with the effect of diets containing levels of the same protein; in which case, the sulfur content of the diets varied directly with the amount of protein in the diet.

The data in Tables II and III tend to indicate a larger proportion of sulfur to nitrogen is required during healing than for normal tissue synthesis. This is supported by the following: (a) after wounding, a positive sulfur balance is observed even when there is a negative nitrogen balance. This may mean that there is a sacrifice of tissue protein nitrogen so that the ratio of sulfur to nitrogen

§ Data represent average urinary output of all animals in these experiments.

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TABLE III. Comparison of Avg Daily Intake and Retention of Dietary Nitrogen and Sulfur and Rate of Healing.*

Group #	Nitrogen: Avg daily intake, mg	Nitrogen: Avg daily balance, mg	Sulfur: Avg daily intake, mg	Sulfur: Avg daily balance, mg	Rate of healing index†
I	266	78	13.2	11.8	16.6
II	78	10	3.6	2.6	10.5
III	269	47	8.2	6.4	12.6
IV	251	56	23.1	14	18.8

* These values indicate daily avg for 21 days after wounding.
† See Fig. 1.

available is increased; (b) increasing the ratio of sulfur to nitrogen by dietary means (by the use of methionine) increases the rate of healing; (c) healing wound tissue contains larger amounts of sulfur than the same tissue when it has not been wounded(21,22).

Thus it seems probable that the rate of healing may be related to, among other factors, the amount of "protein sulfur" retained in excess over that utilized to form normal tissue protein with the retained nitrogen. Since the ratio of nitrogen to sulfur in most tissues is about 16:1, then the healing should be influenced by the amount of retained "protein sulfur," less 1/16 the quantity of retained nitrogen. This might be called the "excess sulfur" which is available for the greater demands of the healing wound tissue. The "excess sulfur" is 156, 44, 75 and 214 mg for the animals in Groups I to IV, respectively. Thus, there appears to be some correlation between the rate of healing and the "excess sulfur" available for the wound.

Summary. Wounded rats were kept on diets containing varying amounts of different proteins and "protein sulfur". There appears to be no relation between the amount of protein nitrogen fed or retained by the rats and the rate of healing. However, the "protein sulfur" retention appears to be correlated with the rate of healing. It is suggested that the amount of retained sulfur in excess over that utilized for normal tissue protein synthesis is an important factor in determining the rate of healing of experimental wounds.

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Variations in Proteolytic Activity of Serum of Animals Including Man.* (18760)

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In a previous publication(1) it was shown that there were significant differences in the activation of plasminogen to plasmin, by streptokinase and protamine, in seven species of animals. It was shown that in man only was there a significant increase of streptokinase activated plasmin over the spontaneously and protamine activated plasmin. In monkeys also there was probable though lesser activation by both streptokinase and protamine. It was also observed that the spontaneous activity in some animals was almost as great as the maximal activation by streptokinase in humans.

With this type of reaction it was necessary

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