

Effect of Sulphur-Amino Acid Deficiency on Wound Healing.*

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From the results of his numerous plant and animal experiments, Hammett^{1, 2, 3} arrived at the conclusion that the sulphhydryl radicle, naturally present in all cells, was the essential and universal stimulus to growth by increase in the number of cells. This hypothesis did not long remain unchallenged. Others,^{4, 5} after carrying out similar investigations, have either not been able to reproduce Hammett's results, or have preferred to lend to them another interpretation. Reimann^{6, 7, 8} translated Hammett's work into the clinic and reported instances of chronic indolent ulcers which healed with dramatic swiftness following the direct application of sulphhydryl compounds. He described a rapid proliferation of both the fibroblastic and epithelial elements of the wound.

In recent years Rose^{9, 10} has demonstrated methionine and not cystine to be the indispensable sulphur-containing amino-acid. Cystine stimulated growth only in the presence of methionine. In the absence of the latter from the ration, however, the animals rapidly lost weight and died, even though an abundant amount of cystine had been supplied.

The purpose of these experiments was to determine whether a deprivation of cystine and methionine in a degree sufficient to abruptly arrest growth, had any effect on the healing of soft tissue wounds. The tensile strength of the wound was used as an index of healing. Young growing rats weighing between 90 and 100 g were divided

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1 Hammett, F. S., *Protoplasma*, 1929, **7**, 297.

2 Hammett, F. S., and Reimann, S. P., *J. Exp. Med.*, 1929, **50**, 445.

3 Hammett, F. S., *Protoplasma*, 1931, **13**, 331.

4 Hueper, W. C., *Arch. Path.*, 1934, **17**, 218.

5 Hueper, W. C., Allen, A., Russel, M., Woodward, G., and Platt, M., *Am. J. Cancer*, 1933, **17**, 74.

6 Reimann, S. P., and Hammett, F. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1929, **27**, 20.

7 Reimann, S. P., *J. A. M. A.*, 1930, **94**, 1369.

8 Reimann, S. P., *Ann. Surg.*, 1931, **93**, 624.

9 Rose, W. C., *Science*, 1937, **86**, 298.

10 Rose, W. C., *Physiol. Rev.*, 1938, **18**, 109.

into 2 groups: (1) normal control, (2) sulphur-amino-acid deficient. The experimental diet used was that described by Dyer and du Vigneaud,¹¹ and consisted of:

Casein	6.0
Dextrin	37.0
Sucrose	15.0
Lard	19.0
Cod liver oil	5.0
Salt mixture	4.0
Agar	2.0
Milk vitamin concentrate	12.0

This is a full diet, adequate in all the essential constituents except the sulphur-bearing amino-acids. It contains cystine and methionine sufficient only for the maintenance of the animal but not for normal growth and increase in weight.

I. Control group—20 animals. This group was maintained throughout the duration of the studies on the experimental diet supplemented with 0.6 g of crystalline cystine. The animals continued to grow and gained an average of one gram in weight per day. Those which did not gain weight were discarded. On the 10th day a longitudinal wound measuring about one cm in length was made under ether anesthesia through the anterior wall of the stomach at its cardiac end. The wound edges were immediately reapproximated in one layer with a running continuous Connell suture of No. 000 plain catgut which, as has been previously shown, loses its tensile strength well within the fourth day.¹² The abdominal wall was closed with 2 layers of fine No. A silk. Strict aseptic precautions were observed. On each of the 4th, 6th, 8th, 10th, and 12th postoperative days 4 animals were sacrificed, and the strength of the wound immediately determined by distending the stomach with air and noting its bursting point. The details of this method were described by Harvey and Howes.¹³ Inasmuch as it has been shown that for approximately 4 days after the injury, the wounds had only the strength contributed by the holding power of the sutures, no studies were made during this first phase of healing.

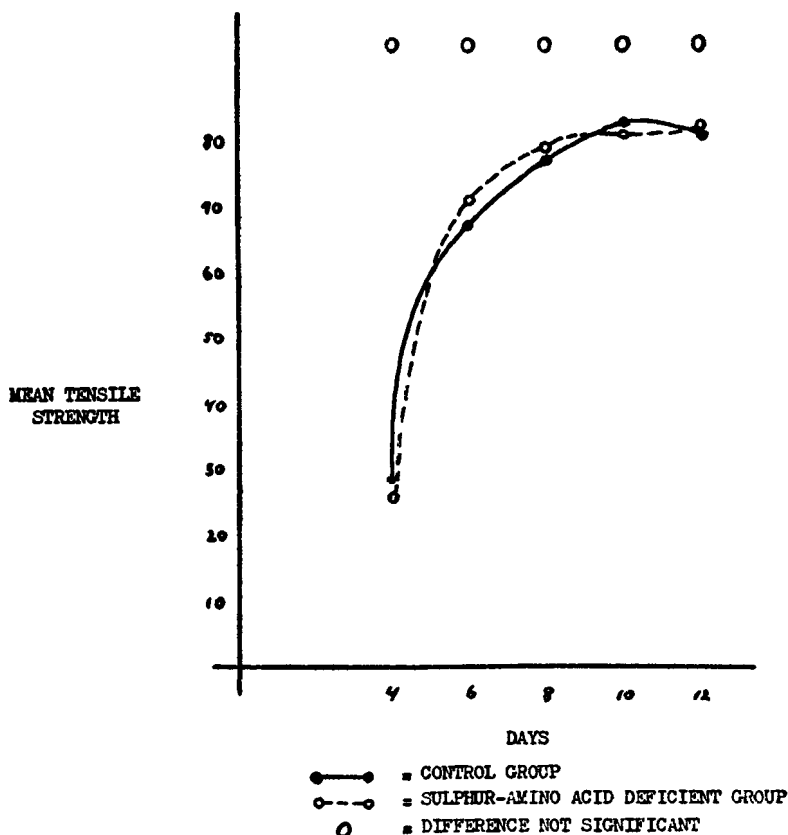
II. Sulphur-amino-acid deficient group—30 animals. This group was maintained on the experimental diet alone. All the animals abruptly ceased to grow. Some slowly lost weight but all survived throughout the duration of the experiment. Although pure amino-acid mixtures were not used, the survival of the animals in this

¹¹ Dyer, H. M., and du Vigneaud, *J. Biol. Chem.*, 1935, **109**, 477.

¹² Howes, E. L., *J. A. M. A.*, 1928, **90**, 530.

¹³ Harvey, S. C., and Howes, E. L., *Ann Surg.*, 1930, **91**, 641.

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GRAPH I.

group and their failure to grow indicate, in an otherwise complete ration, a deficiency of methionine as well as cystine. On the 10th day, wounds exactly similar to those of the control group were made. Determinations of the tensile strength were carried out from the 4th through the 12th post-operative day.

In one of the members of the control group a small walled off abscess was found adjacent to the operative site in the stomach. This animal was discarded. In the remaining 49 animals of both series, the stomach and abdominal wounds all healed *per primam*. No troublesome adhesions were encountered. In each group the averages of the breaking strength for every postoperative interval, as well as the standard deviation, were computed. Fisher's¹⁴ formula for small samples was applied to determine whether the difference in the means of the two groups was statistically significant.

¹⁴ Fisher, R. A., *Statistical Methods for Research Workers*, Edinburgh, 1934.

Results. No significant deviation from the normal healing of stomach wounds was discovered in young rats maintained upon a ration deficient in cystine and methionine. The curves of healing of these wounds, as measured by their tensile strengths, were practically identical in the control and in the experimental groups. (Graph I.) (The distended stomachs ruptured at places elsewhere than the operative sites in 10 of the 11 control animals, and in 15 of the 17 experimental animals for the 8-, 10- and 12-day periods.) These findings do not warrant any opinion concerning the allegedly essential rôle of sulphhydryl compounds as the real stimuli to cell growth and proliferation. Nor can any conclusions be justly drawn regarding the absolute indispensability of the sulphur-bearing amino-acids in the process of fibroblastic proliferation and wound healing. If these substances are indeed essential in this process, then it must be postulated that the animal, not provided with enough of the sulphur compounds in its diet to allow adequate body growth, is still able to derive from some other source a quantity sufficient to permit adequate wound healing. This source is possibly endogenous and may be related to the breakdown of its own body proteins.

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Biological and Immunological Identity of *Toxoplasma* of Animal and Human Origin.

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There exists considerable confusion among parasitologists as to the characteristics which justify the classification of Protozoa of certain morphology as *Toxoplasma*. The capacity to multiply and to produce disease in a variety of hosts, including mammals and birds, must, in accord with the conclusions of Aragao,¹ be regarded as the chief taxonomic characteristics of the group. Morphology as the only guide can be misleading and confusing as is evident from the present controversy as to whether certain forms observed in avian malaria are *Toxoplasma* or stages of *Plasmodium*.² In accord with Aragao's criteria, the existence of *Toxoplasma* in North America was first demonstrated by Sabin and Olitsky,³ who isolated *Toxo-*

¹ Aragao, H. de B., *Compt. rend. Soc. biol.*, 1933, **113**, 214.

² Hegner, R., and Wolfson, F., *Am. J. Hyg.*, 1938, **27**, 212; *ibid.*, 1938, **28**, 437.

³ Sabin, A. B., and Olitsky, P. K., *Science*, 1937, **85**, 336.