Some Effects of Sulfur Amino Acids upon Tumor-Host Relationship in the Rat*

RUSSELL HILF†

(Bureau of Biological Research and Department of Physiology and Biochemistry, Rutgers University, New Brunswick, N.J.)

Previous studies have demonstrated that a semisynthetic diet containing 12 per cent casein is deficient in sulfur amino acids for the growth of tissues in rats (1), supplementation with 0.7 per cent DL-methionine increasing the growth optimally. A transplanted sarcoma was found to develop most rapidly in rats fed this sulfur amino acid-deficient diet. Supplementing with 0.7 per cent methionine increased the growth of normal tissues and depressed the development of the tumor (4). Adding more nitrogen in the form of guanidoacetic acid (glycocyamine) or glycine increased the growth of the normal tissues still further and tended to depress the development of the tumor. The effect of glycine and guanidoacetic acid seemed to be specific and not just the result of adding more nitrogen. The mixture of methionine and guanidoacetic acid was particularly effective in conserving body nitrogen under conditions of stress such as the depleting effect of a large sarcoma or of the chemotherapeutic agent TEPA (3).

Further experiments are reported here to determine the optimum amounts of methionine for supplementation in tumor-bearing animals and to compare the supplementing effects of cystine with methionine. Furthermore, the best proportions for a mixed supplementation of methionine plus guanidoacetic acid were sought. Food efficiencies were used as well as gain in body weight with respect to tumor weight to determine the effects of supplementation.

MATERIALS AND METHODS

Male Wistar rats, weighing between 125 and 150 gm, in groups of ten, were fed a semi-synthetic diet (12 per cent casein) as described previously by Allison et al. (4). All supplements are expressed as percentage of dry weight components. The feeding of the diets was started at the time of transplantation, all rats being fed ad libitum, and daily food intakes were recorded. From these data, food efficiency was calculated. The term “food efficiency” is defined here as the gain in body weight (total animal weight — tumor weight) per 100 grams of food eaten.

The tumor used in these experiments was the Sarcoma R-1, which arose spontaneously from mammary tissue. After a number of successive transplantations, this tumor has resolved into a sarcoma, the characteristics of which will be described in a separate publication. The tumor was homogenized in a loosely fitting glass homogenizer to which penicillin (2000 units/gm tissue) was added. Each animal received 0.12 gm. of homogenized tissue transplanted subcutaneously in a lateral position.

The length and average diameter of the tumors were measured at various intervals with a caliper, and the weight of the tumor was calculated in vivo according to the following formula: $0.796 \times a \times b \times \text{specific gravity of the tumor}$, where $a$ equals the mean width and $b$ equals the length of the tumor. The specific gravity of this sarcoma was determined to be 1.023, and calculations of tumor weights with this formula resulted in excellent correlations (4). Tumor weights were subtracted from the total weight of the rat to determine body weight.

Experiments demonstrated that this sarcoma exhibited a two-phase growth, the first being devoted to establishment and the second to rapid growth. The number of days for the tumor to reach 1 gm. was called the induction period, such a tumor being well established. A ratio was used as an indication of the over-all effect of the diet. This ratio, $\Delta \text{body weight/tumor weight}$ represents the change in body weight with respect to the tumor weight after the induction period. Thus, the smaller the ratio, the more depleted the host.

RESULTS AND DISCUSSION

The sarcoma developed to lethal proportions of approximately 20 gm. over a period of 2 weeks.
The data illustrated in Chart 1 demonstrate that supplementing the 12 per cent casein diet with from 0.2 to 1.0 per cent DL-methionine reduced the size of the tumor during this growth period of 2 weeks. At the same time, the body of the animal (total weight of tumor-bearing animal — tumor) grew maximally in animals fed the diet supplemented with 0.2–0.7 per cent DL-methionine, with some evidence for minimum development of the tumor and maximum growth of the body when the 12 per cent casein diet contained 0.7 per cent DL-methionine. This amount of the amino acid has been found to be optimum also for the growth of normal rats (1). A similar depression in the development of the sarcoma and improved growth of the body of the rat were obtained by supplementing the diet with 0.6 and 1.0 per cent cystine. These studies and those published previously have indicated that the decreased development of the sarcoma in the presence of the supplemented diets is the result in part of a longer induction period leading up to the rapid growth phase. The improvement in growth of the normal tissues, however, can also reduce the rate of growth of the tumors at all stages (8).

The improved growth of the normal tissues in sarcoma-bearing rats fed the supplemented diets is revealed also by improved food efficiencies (gm gained in body weight/100 gm of food eaten, body again referring to total weight less tumor weight). The food efficiency for animals fed the 12 per cent casein diet over the period of 2 weeks was 10.5. Supplementing with the following amounts of methionine (0.2, 0.4, 0.7, and 1.0 per cent) resulted in these food efficiencies: 14.8, 14.7, 15.9, and 13.0, respectively. Similarly, supplementing with 0.3, 0.6, 1.0, and 1.5 per cent cystine gave the following food efficiencies: 11.8, 19.6, 18.4, and 14.8, respectively.

One of the best ways we have found to express the effect of improved growth of normal tissues on the development of this sarcoma has been to use a \( \Delta \) body/tumor ratio. The increase in body weight can be taken during the rapid growth phase of the tumor (from 1 to 20 gm.) when the stress on the body is greatest. This was done to reveal the supplementing effects of additional amounts of nitro-
mentation on \(\Delta\) body/tumor ratios. Addition of guanidoacetic acid to methionine markedly enhanced the growth of normal tissues in the presence of the sarcoma, a ratio of 1:1 (0.7 per cent methionine plus 0.7 per cent glycocyamine) promoting optimal body growth. This, in addition to a suppressed tumor growth, resulted in a significantly higher ratio than any of the other combinations used. In combination with cystine, however, guanidoacetic acid did not enhance body weight significantly. This suggests the possibility of different metabolic pathways when combined therapy is employed. Tumor growth in all cases was reduced below that of control animals, smallest tumor resulting in the group receiving the 1:1 combination of methionine and guanidoacetic acid. Food efficiency data followed the same pattern as body/tumor ratios, maximum value of 18.5 obtained by feeding the basal diet supplemented with 0.7 per cent methionine plus 0.7 per cent glycocyamine as compared with 11.8 for unsupplemented controls and 16.6 for methionine-supplemented animals. The cystine and guanidoacetic acid combination also resulted in an increased food efficiency, maximum value obtained with the 1:1 supplementation group (17.7), but not so high as the comparable methionine group. It is interesting to note that addition of guanidoacetic acid by itself to the basal diet resulted in toxic effects to the sarcoma-bearing animals. Supplementing the 12 per cent casein diet with 0.5, 1.0, and 2.0 per cent glycocyamine resulted in a decreased body growth as the amount of this acid was increased and also caused decreased body/tumor ratios—a decrease of 4 per cent, 25 per cent, and 55 per cent, respectively. Food efficiency data also illustrate the toxic effects of glycocyamine, values of 10.0, 9.3, and 5.5 gm gained/100 gm food eaten as the level of this acid was increased, as compared with 10.5 gm gained/100 gm food eaten for the unsupplemented control animals. However, in combination with sulfur amino acids, this toxicity was not only overcome but the combination resulted in an additive effect.

To determine the specificity of guanidoacetic acid, other sources of nitrogen were substituted on an equal nitrogen basis as compared with the optimal glycocyamine level (0.7 per cent). Results with glycine and ammonium citrate in combination with methionine are shown in Table 1. Tumor weights are reduced by methionine addition as noted previously. \(\Delta\) Body/tumor ratios indicate the effect of combined supplementation upon normal tissue growth. The negative ratio obtained in the unsupplemented 12 per cent casein controls resulted from a loss in body weight (total weight—tumor) during the period in which the tumor rapidly grew from 1 to 17 gm. This is the period of stress in which the tumor grows rapidly at the expense of the normal tissues of the host animal. Methionine can partially overcome this, but combination of methionine with guanidoacetic acid or glycine is far more effective. Ammonium citrate, a nonspecific source of nitrogen, does not enhance the methionine effect. Under additional stress, such as TEPA therapy, glycine is no longer so effective as guanidoacetic acid, and addition of alanine or additional casein produces the same effect as ammonium citrate (2). The food efficiency data correlate the body/tumor ratios in showing the effectiveness of combined supplementation with guanidoacetic acid or glycine.

### SUMMARY

Data have been presented to demonstrate that methionine and cystine reduce the growth of Sarcoma R-1, increase the body weight, increase food efficiency, and increase \(\Delta\) body/tumor ratios. Supplementation with guanidoacetic acid proved to be toxic, this toxicity being overcome when combined with the sulfur amino acids. Guanidoacetic acid or glycine in combination with methionine resulted in increased food efficiency and body/tumor ratios, a response which suggests a specific role for these acids.

### REFERENCES

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Russell Hilf


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