results from a given set of experimental conditions. This factor is of particular significance when one is dealing with pharmacological agents which often produce such a state of severe starvation.

*Summary.* 1. The vitamin $B_C$, pantothenic acid, biotin, riboflavin, pyridoxine, and nicotinic acid contents of the livers, kidneys, and bone marrows have been determined in rats: (a) fed *ad libitum;* (b) treated with mustard gas; and (c) whose food intake was restricted. 2. When expressed on the basis of unit weight of tissue, increases of 50 to 180% were noted in the vitamin concentrations of livers from the food-restricted group. Less marked increases occurred in the kidney, while a decrease of 35% in the vitamin $B_C$ content of the bone marrow based on nitrogen content was observed. 3. With the exception of the vitamin $B_C$ content of the bone marrow, the changes observed in the partially-starved group were also found in the mustard group. 4. Calculations based upon the vitamin content of the liver per 100 g of body weight indicate that both starvation and treatment with mustard gas cause increases ranging from 15 to 130% in the vitamin $B_C$, pantothenic acid, biotin, and pyridoxine contents of the liver. No significant changes occur in the riboflavin or nicotinic acid contents.

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## Toxicity of Amino Acids as Influenced by Riboflavin Deficiency.

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The therapeutic application of protein hydrolysates has become common and as a result it grows more and more important to understand interrelationships between amino acids, vitamins, minerals, etc. In the course of experiments involving toxicity studies of histidine[1] and tyrosine,[2] it became probable that riboflavin deficiency was a key to increased amino acid toxicities. To establish this point, histidine and tyrosine toxicities were rechecked and cystine, glycine, glutamic acid and tryptophane were investigated.

*Experimental.* The various amino acids were incorporated into a synthetic diet[3] at varying levels from 5 to 20% depending upon the relative toxicity of the amino acid under consideration. The amino acid replaced an equivalent amount of carbohydrate in the basic diet. Glycine, *l*-tyrosine, *l*-histidine, *l*-cystine, *d*-glutamic acid and *dl*-tryptophan were used. The rats in all series showed crusted and bloody snouts, spectacled eyes, and diarrhea. The animals receiving glycine with or without superimposed riboflavin deficiency lost their hair; some became almost hairless. The rats were lethargic and appeared to lack muscular coordination. In those receiving glycine without a superimposed $B_2$ deficiency the hair condition corrected itself after a few weeks. Emaciation was common.

The symptomatology seen in these rats on toxic levels of an amino acid is also seen in riboflavin deficiency[4] but it should be recalled that these manifestations are nonspecific in nature.

Table I summarizes our results.

Accentuation of the toxicities of amino

1 Martin, G. J., in press.

2 Martin, G. J., in press.

3 Martin, G. J., *Arch. Biochem.*, 1943, **1**, 397.

4 McCollum, E. V., Orent-Keiles, E., and Day, H., *Newer Knowledge of Nutrition*, 1939, Macmillan, New York.

TABLE I.
Toxicity of Amino Acids as Influenced by Riboflavin Deficiency.
10 rats to each set.

| Diet | % dead Months 0 | 1 | 2 | Avg wt, g Months 0 | 1 | 2 |
|---|---|---|---|---|---|---|
| 5% Tyrosine | 0 | 0 | 0 | 47 | 84 | 162 |
| " " Riboflavin def. | 0 | 70 | 100 | 46 | 60 | 70 |
| 10% Histidine | 0 | 30 | 40 | 46 | 68 | 82 |
| " " Riboflavin def. | 0 | 80 | 100 | 42 | 63 | — |
| 5% Cystine | 0 | 0 | 20 | 51 | 102 | 173 |
| " " Riboflavin def. | 0 | 60 | 80 | 48 | 78 | 64 |
| 20% Glycine | 0 | 20 | 40 | 50 | 74 | 98 |
| " " Riboflavin def. | 0 | 60 | 100 | 53 | 46 | — |
| 10% Glutamic acid | 0 | 20 | 40 | 48 | 115 | 198 |
| " " " Riboflavin def. | 0 | 20 | 20 | 47 | 78 | 84 |
| 20% *dl*-tryptophane | 0 | 20 | 40 | 42 | 75 | 63 |
| " " Riboflavin def. | 0 | 20 | 80 | 49 | 41 | 32 |
| Riboflavin def. | 0 | 0 | 10 | 41 | 78 | 89 |

acids occurred without exception in the 6 instances reported. This is established both by the increased death rate and by the decreased growth responses seen in the riboflavin-deficient animals. Tyrosine (5%), histidine (10%), and glycine (20%) were the most toxic amino acids in the riboflavin-deficient animals; whereas histidine (10%), glycine (20%), glutamic acid (10%) and tryptophan (20%) were the most toxic in the rats receiving riboflavin.

*Discussion.* The toxicity of tyrosine,[3,5] histidine,[2,6] glycine,[7] tryptophan,[5] cystine,[7] lysine,[5] serine,[8] methionine,[9] leucine,[6] hydroxyproline,[6] aspartic acid[6] has been reported. Glutamic acid is now added to this list. The results in this paper indicate increased toxicities for glycine, tryptophan, glutamic acid, cystine, histidine and tyrosine in the presence of a riboflavin deficiency. Balance is the deciding factor but there is specificity in these balance relationships as shown by the increased toxicity of tyrosine in a riboflavin deficiency[1] with no similar result in thiamin, pantothenic acid, folic acid, pyridoxine and vitamin A and D deficiencies. It is reflected in the ability of certain amino acids to counteract increased toxicities while others do not. As an example of this cystine decreased the toxicity of tyrosine,[1] while tryptophan and glutamic acid have no effect.

The explanation offered for consideration in the specific action of riboflavin deficiency in increased amino acid toxicities is that *l*-amino acid oxidase probably is a riboflavin-containing enzyme.[10]

*Summary.* *l*-Cystine, *dl*-tryptophan, *l*-tyrosine, *l*-histidine, glycine, and *d*-glutamic acid are more toxic in the riboflavin-deficient rat than in the rat receiving this vitamin.

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6 Graham, C. E., Hier, S. W., and Klein, David, *Abstracts 110th Meeting A. C. S.*, 1946, 7K.

7 Curtis, A. C., and Newburgh, L. H., *Arch. Int. Med.*, 1924, **29**, 828.

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9 Earle, D. P., Smull, K., and Victor, J., *J. Exp. Med.*, 1942, **76**, 317.

10 Ratner, S., Nocito, V., and Green, D. E., *J. Biol. Chem.*, 1944, **152**, 119.