9820 P

Amino-Acids in Staple Foods. The Rôle of Cystine in Proteolytic Digestion.

FRANK A. CSONKA.

From the Protein and Nutrition Research Division, Bureau of Chemistry and Soils, United States Department of Agriculture.

The author presented his views on the synthesis of amino-acids in the animal body at the Washington annual meeting of the Society of Biological Chemists in 1936 (unpublished) and concluded that cystine, contrary to the view held at that time, is a dispensable amino-acid. His working hypothesis was that sugar forming amino-acids are dispensable and non-sugar formers are indispensable, and also suggested that valine being a non-sugar former would prove to be an indispensable amino-acid. There are so many examples in the literature where the diet under investigation was supplemented with cystine which promptly converted it to a normal growth-producing one that a claim of cystine deficiency was readily granted. Johns and Finks[1] fed cooked navy bean meal supplemented with cystine to rats and obtained normal growth so that they concluded that cystine deficiency was the controlling factor in the bean diet. Mitchell and Smuts'[2] feeding experiments demonstrated clearly that by supplementing raw soybean meal with cystine the rats not only stayed alive but gained in weight. Csonka and Jones[3] stated, however, that there cannot be a quantitative deficiency in soybean meal when the defatted meal contains an average of 0.4% cystine. Regardless of the cystine content of the experimental diets of soybean (high) and navy bean (low) the addition of cystine produced growth. Since the dispensability of cystine to growth has been now demonstrated by Rose and coworkers[4] experimentally, we are forced to assume that supplementary cystine performs some physiological function other than correcting a nutritional deficiency. There is some justification for considering first that free -SH has an activating effect upon proteolytic enzymes and, second, that the oxidation-reduction properties of this cystine (or cysteine) may affect digestion favorably. The former creates an oxidizing medium which is conducive,

[1] Johns, C. O., and Finks, A. J., *J. Biol. Chem.*, 1920, **41**, 379.

[2] Mitchell, H. H., and Smuts, D. B., *J. Biol. Chem.*, 1932, **95**, 263.

[3] Csonka, F. A., and Jones, D. B., *J. Agric. Res.*, 1934, **49**, 279.

[4] Rose, W. C., *Science*, 1937, **86**, 298.

according to Voegtlin, Maver, and Johnson,[5] to synthesis of proteins, while the latter imparts a reducing medium favorable to hydrolysis. It was found by the author that the addition of cystine or cysteine to navy bean meal has an accelerating effect on peptic and tryptic digestion *in vitro*. The acceleration of proteolytic enzyme activity is more pronounced with cysteine supplementation when the substrate is in the raw state; when the cooked bean meal is digested, cystine is found more effective. A quicker and higher degree of digestion may result in a better assimilation and utilization of the bean protein by the animal body, which results in increased body weight, the improvement noted in feeding experiments mentioned above.

The cystine present in peptide linkage in bean seed proteins apparently is not in available form; free cystine is not formed by digestion with pepsin or trypsin. Acid hydrolysis of a pepsin digest showed that all the cystine present in the seed originally was accounted for, but acid hydrolysis of a trypsin digest showed no detectible cystine by Sullivan's method. These experiments were carried out on defatted soybean meal in 1934.

Autolysis of the raw bean meal in (pH 5) disodium citrate solution is also accelerated by the addition of H_2S, cysteine or cystine in the order mentioned, which suggests the presence of a plant pepsin in the navy bean seed.

TABLE I.

Digestion Experiments with Raw Navy Bean Meal. Incubation Temperature 38°C.

		Autolysis		Pepsin		Trypsin	
	Type of N	3 hr.	72 hr.	3 hr.	72 hr.	3 hr.	72 hr.
Raw navy bean meal	Amino-acid	1.47	4.41	4.2	5.6	11.2	15.4
	Polypeptide	0.63	3.99	0	3.0	3.8	7.6
50 mg. cysteine supplement	Amino-acid	0	2.94	7.0	8.4	9.8	19.6
	Polypeptide	1.05	4.61	3.6	8.2	10.2	10.4
50 mg. cystine supplement	Amino-acid	3.15	5.88	7.0	7.0	12.6	16.8
	Polypeptide	0	3.57	0.6	5.6	3.4	8.2
H_2S	Amino-acid	2.94	1.47				
	Polypeptide	0.21	4.83				

To show the magnitude of acceleration caused by cystine or cysteine supplementation, digestion experiments on raw navy bean are tabulated in a condensed form. In the pepsin digestion 20 cc. of 0.05 N HCl containing 0.2 gm. of pepsin (Merck) was added to 2 gm. of navy bean meal containing 75 mg. N., while in the trypsin

[5] Voegtlin, C., Maver, M. E., and Johnson, J. M., *J. Pharm. and Exp. Therap.*, 1933, **48**, 241.

experiments the 2 gm. navy bean meal was suspended in 20 cc. of disodium phosphate solution of pH 8.9 containing 0.2 gm. of trypsin (Fairchild's). Willstätter and Waldschmidt-Leitz's[6] titration method was used and the figures represent increases over those obtained at the beginning, expressed in cubic centimeters of 0.1, normalcy representing 1.4 mg. amino N per cc. In pepsin digestion changes in the polypeptide N and in trypsin digestion, changes in the amino-acid N served as a measure of proteolytic acceleration.

9821 P

Investigations on the Pathogenesis of Tetanus.

BERNARD ZUGER AND ULRICH FRIEDEMANN. (Introduced by Benjamin Kramer.)

From the Division of Bacteriology, Jewish Hospital of Brooklyn.

We have observed that 10 to 20 times more tetanus antitoxin given intravenously is required to protect guinea pigs and rabbits against a multiple lethal dose of the toxin injected intramuscularly than intravenously. Table I gives the results of a representative experiment.

TABLE I.

Comparison of the Amounts of Antitoxin Required to Protect Guinea Pigs against 10 Fatal Doses of Tetanus Toxin Injected Intramuscularly and Intravenously.
Tetanus Toxin—N. Y. City Board of Health Laboratories Lot 47 Vial C.
Antitoxin " " " " " " Lot 284.

Antitoxin 1 cc. intravenously, dilutions	Toxin 0.1 cc. 1:200			
	G.p. No.	Intramuscularly	G.p. No.	Intravenously
1:50	507	LT, partial		
1:100	506	LT, "		
1:200	505	LT, complete		
1:500	504	LT, "		
1:1000	503	LT, "		
1:2000	502	LT, GT *3		
1:4000	501	LT, GT *6		
1:5000			511	0
1:10,000			510	0
1:20,000			509	0
1:40,000			508	GT *5

LT—local tetanus.
GT—generalized tetanus.
0—no symptoms.
*—death, number following indicating day after injection.

[6] Willstätter, R., and Waldschmidt-Leitz, E., *Berichte*, 1921, **54**, 2988.