16652

Effect of Amino Acid Deficiencies on Incorporation of Radioactive Carbon-Labeled Amino Acids into Animal Tissue Proteins.*

DATTATREYA RAO SANADI† AND DAVID M. GREENBERG.

From the Division of Biochemistry, University of California Medical School, Berkeley.

Comparatively little is known of the effect of malnutrition on the metabolic processes of the organism. Accumulation of such information is of importance for the advancement of biochemistry. In the present investigation it is shown by means of isotopic tracer experiments that deficiency of either of the essential amino acid tryptophan or phenylalanine results in impaired ability of the mammalian organism to incorporate C^{14} from labeled amino acids into protein.

The introduction of protein-hydrolysates in therapy has led to the development of the concept that all essential amino acids must be present simultaneously for protein synthesis and growth. The injection of tryptophan a few hours after the injection of a casein-hydrolysate containing no tryptophan failed to induce positive nitrogen balance, while simultaneous administration of the two was successful.[1] Melnick and coworkers[2] attributed the lower biological value of soy protein compared to that of heat-processed soy protein, to a delayed liberation of methionine during digestion in the gastrointestinal tract. When an incomplete mixture of amino acids was fed to rats and the missing essential amino acid supplemented after an interval of several hours, the animals did not grow.[3] It was of interest, therefore, to study the behavior of a tracer dose of a labeled amino acid in an essential amino acid deficiency to obtain information on the effect of the malnutrition on the C^{14} uptake by the tissue proteins of the organism.

Young male rats (Long Evans strain) were placed on a tryptophan deficient diet.[4] Another litter-mate group received the same diet with 0.5% L-tryptophan added. After about 8 weeks, when the symptoms of deficiency had developed, DL-tryptophan-β-C^{14}‡ (0.85 μ C per mg or 105,000 counts per minute per mg) was injected intraperitoneally (3 mg per 100 g body weight). The animals were then fasted for 8 hours and sacrificed. Protein samples from different tissues were prepared for radioactivity assay by homogenizing the tissues and precipitating and washing the protein with trichloracetic acid followed by washings with hot alcohol, alcohol-ether mixture and ether. The radioactivity counts were corrected for self absorption from a curve prepared by counting a highly radioactive sample of protein in layers of different thickness.

In another series of experiments, phenylalanine deficiency was produced in male mice on a synthetic diet,[5] the control group receiving in addition 1.2% DL-phenylalanine. After 3 to 4 weeks, one set of mice received intraperitoneally (3 mg per 100 g body weight) carboxyl-labelled glycine (5.37μc/mg) and another set received labeled tryptophan (2 mg/100 g). The animals were killed 8 hours after injection as in the previous experiment, but in this case they had free access to food. The proteins of the tissues were treated as before.

It is seen from the data (Table I) that the incorporation of the label into the tissue proteins is reduced in essential amino acid de-

* Aided by grants from the Rockefeller Foundation and the Research Funds of the Medical School.

† Indian Government scholar.

1 Elman, R., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 484.

2 Melnick, D., Oser, B. L., and Weiss, S., *Science*, 1946, **103**, 326.

3 Geiger, E., *J. Nutr.*, 1947, **34**, 97.

4 Berg, C. P., and Rose, W. C., *J. Biol. Chem.*, 1929, **82**, 479.

‡ Synthesized by Dr. Charles Heidelberger and supplied through the generosity of Dr. Heidelberger and Dr. Melvin Calvin.

5 Womack, M., and Rose, W. C., *J. Biol. Chem.*, 1947, **171**, 37.

TABLE I.
Activity of C^{14} in Tissue Proteins in Counts per mg per Minute.*

	Tryptophan deficiency		Phenylalanine deficiency			
	C^{14}-Tryptophan administered		C^{14}-Tryptophan administered		C^{14}-Glycine administered	
Organ	Supplemented	Deficient	Supplemented	Deficient	Supplemented	Deficient
Intestines	64.8	28.9	43.9	38.8	197	174
Plasma	52.0	28.0				
Liver	41.5	27.9	20.3	17.7	140	105
Kidney	34.9	21.4	29.7	20.6	118	68.0
Spleen	22.7	9.6	28.2	17.5	114	90.2
Testes	19.6	5.4				
Stomach	30.3	8.0				
Heart	10.5	4.3	8.4	5.1	43.0	29.4
Brain	10.2	4.3				
Skeletal muscle	4.7	2.7	4.5	1.8	23.4	5.0
Red cells	5.0	2.0				

* The data represents the average value of 2 animals in each group.

ficiency both when the animals are starved or fed. The decrease may be due to a decrease in net synthesis of protein or to a lowered rate of turnover, or to both combined. The deficiency of the amino acid could directly limit the quantity of enzymes responsible for the incorporation of the label or the effect may be an adaptive response.

Summary. Animals reared on a diet deficient in an essential amino acid (tryptophan, phenylalanine) showed a reduced incorporation of tracer amounts of C^{14} from labeled amino acids in the tissue proteins.

16653

Passive Transfer of Local Cutaneous Hypersensitivity to Tuberculin.

M. N. METAXAS AND M. METAXAS-BÜHLER. (Introduced by Merrill W. Chase.)
From the Hygiene Institute of the University of Zürich, Switzerland.

Many attempts have been made in the past to transfer tuberculin hypersensitivity passively to normal animals. Most of these attempts have been unsuccessful, and the positive results claimed by some authors have either been seriously contested by others, or have been too irregular to be quite convincing.[1,2] A method which insures a readily reproducible transfer of tuberculin hypersensitivity was first described by Chase[3]; this method had been previously devised by Landsteiner and Chase[4] for the passive transfer of the "delayed type" of skin sensitivity to simple chemical compounds. Briefly, it consists in injecting a suspension of living exudate, spleen or lymph node cells from actively sensitized guinea pigs into the peritoneal cavity or the blood stream of normal animals, following which the latter develop general cutaneous hypersensitiveness to tuberculin within 1 to 3 days. Using the same or slightly modified methods, Kirchheimer and Weiser,[5] Cummings, Hoyt, and Gottshall,[6]

1 Zinsser, H., and Mueller, J. H., *J. Exp. Med.*, 1925, **41**, 159.

2 Rich, A. R., The Pathogenesis of Tuberculosis, p. 398, 2nd printing, Springfield, Charles C. Thomas, 1946.

3 Chase, M. W., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 134.

4 Landsteiner, K., and Chase, M. W., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 688.

5 Kirchheimer, W. F., and Weiser, R. S., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 166.