Protein Synthesis in Liver of Pantothenic Acid-Deficient Rats[1] (35993)

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Previous studies of Axelrod and co-workers have demonstrated that a pantothenic acid deficiency in rats causes a severe impairment of antibody response [(1–3), Lederer, Kumar, and Axelrod (unpublished observations)]. The role of this vitamin in the development of the immune process has been reviewed (4). In order to investigate the relationship between pantothenic acid (PA) deficiency and the synthesis of other serum proteins, we examined the *in vivo* incorporation of ^{14}C-amino acids into circulating serum albumin. The results of such experiments demonstrating a decreased incorporation of labeled amino acids into the serum albumin fraction of PA-deficient rats and our subsequent attempts to establish a subcellular basis of this observation are presented below.

Materials and Methods. Animals and diets. Male rats (19 days old) of the Holtzman strain were utilized. The animals were housed individually in wide-meshed, screen-bottom, suspended cages. Composition of the purified basal diet fed to both the control and the PA-deficient rats has been described previously (2). In addition, each rat received daily a vitamin pill which, for control animals, contained adequate quantities of the B vitamins (2) including 0.2 mg of calcium pantothenate; PA-deficient rats received the same pill devoid of pantothenic acid. All animals were fed the basal diet *ad libitum* and were weighed weekly. After 11 weeks, rats in the PA-deficient group received 7 daily ip injections of normal saline solutions of ω-methyl pantothenic acid[3] (10 mg/ml) at a dosage of 2 mg/100 g of body weight. Experimental procedures were instituted at the conclusion of this 12-week period. At this time, the body weights of the control rats ranged between 250 and 300 g (av, 285 g), while those of the PA-deficient rats ranged between 150 and 200 g (average, 160 g).

In vivo *amino acid incorporation.* A ^{14}C-amino acid mixture[4] (1.5 mCi/mg in 0.1 *N* HCl), whose pH was adjusted to 6.0 with solid Tris buffer before use was administered ip to control and PA-deficient rats at a level of 5 μCi/100 g of body weight. Blood samples were taken at varying time intervals through cardiac puncture under light ether anesthesia and the serum albumin fraction obtained by the trichloroacetic acid–ethanol method of Debro *et al.* (5). After dialysis of the trichloroacetic acid–ethanol extract against water, aliquots were removed for determination of protein concentration by the method of Lowry *et al.* (6). An aliquot (0.2 ml) of this dialyzed extract was mixed with 5 ml of Bray's fluid and radioactivity was determined in the Packard liquid scintillation spectrometer (7).

Polysomal analysis. Livers were quickly removed from rats under light ether anesthesia and polysomal analysis of the postmitochondrial supernatant was performed according to the method of Wettstein *et al.* (8). Equal OD_{260} units of the postmitochondrial supernatants were layered directly on an exponentially convex sucrose gradient (0.3–1.0 *M*) after treatment with 1.3% sodium deoxycholate (DOC). After centrifugal sepa-

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[3] Purchased from the California Foundation for Biochemical Research.

[4] Purchased from the New England Nuclear Corporation.

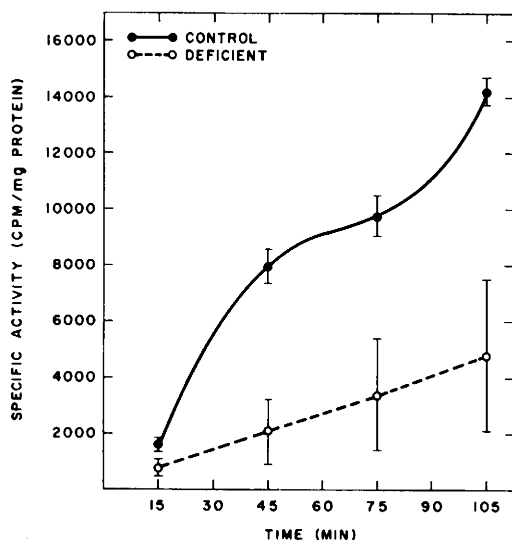

Fig. 1. Time course of appearance of radioactivity in circulating serum albumin in pantothenic acid-deficient and control rats after ip administration of ^{14}C-amino acids: Each of 4 pantothenic acid-deficient and 4 control rats was bled serially at 15, 45, and 75 min after injection of labeled amino acids. Serial bleedings at 15, 45, and 105 min were performed in another set of 4 pantothenic acid-deficient and 4 control rats. Each point represents average values ± SE.

ration, the samples were passed through an automatic optical recording unit connected to a fraction collector. Labeling of the nascent peptides associated with the polysomes was achieved by a 3-min intravenous pulse of the ^{14}C-amino acid mixture administered via a tail vein at a level of 5 μCi/100 g of body weight. After centrifugation, samples (1 ml) were collected as described, mixed with 10 ml of Bray's fluid, and radioactivity was assayed in a Packard liquid scintillation spectrometer (7).

In vitro *amino acid incorporation.* For the preparation of microsomes and the supernatant fractions, livers were quickly removed from rats under ether anesthesia and placed into ice-cold 0.25 *M* sucrose in TMK buffer (0.05 *M* Tris HCl, pH 7.6; 0.004 *M* magnesium acetate; 0.025 *M* KCl). Finely minced liver was then homogenized with a Teflon–glass, Potter-Elvehjem type tissue homogenizer in 3 vol of .25 *M* sucrose in TMK buffer. The homogenate was centrifuged twice at 13,000 rpm for 20 min to obtain the postmitochondrial supernatant (PMS). Centrifugation of the PMS at 40,000 rpm for 1 hr yielded a microsomal pellet and the supernatant enzyme fraction. Microsomes were suspended in 0.25 *M* sucrose and centrifuged at 5000 rpm to remove any clumps. Protein concentrations of the microsomal suspensions and the supernatant enzyme fractions were assayed by the method of Lowry *et al.* (6). The incubation mixture for the *in vitro* amino acid incorporation contained: adenosine triphosphate, 2 μmoles; guanosine triphos-

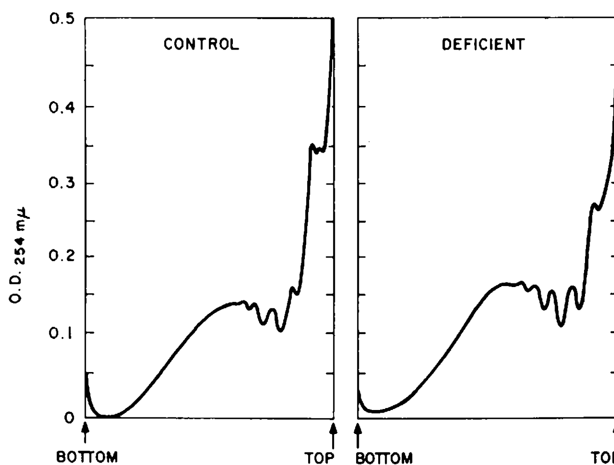

Fig. 2. Representative hepatic polysomal profiles of 5 pantothenic acid-deficient and 5 control rats. Variations between animals of each group were not significant and only representative data are presented.

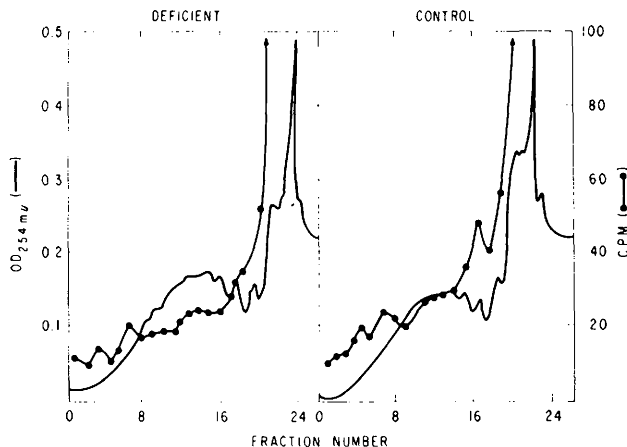

FIG. 3. Patterns of labeled nascent peptides associated with polysomes of 5 pantothenic acid-deficient and 5 control rat livers. Variations between animals of each group were not significant and only representative data are shown. Arrows indicate radioactivity beyond the scale due to the high activity of the soluble proteins at the top of the gradient.

phate, 1 μmole; phosphoenolpyruvate, 10 μmoles; phosphoenolpyruvate kinase, 80 μg; ^{14}C-amino acid mixture, 1.25 μCi; penicillin, 100 units; microsomal suspension (3 mg of protein); and supernatant enzyme fraction (7 mg of protein). The final volume was adjusted to 1.0 ml with TMK buffer. After incubation at 37°, the reaction was stopped with 1.0 ml of 20% trichloroacetic acid (TCA); and the precipitate was washed 3 times with 5.0 ml of 10% TCA. The precipitate was then suspended in 5.0 ml of 10% TCA and incubated at 90° for 15 min. The tubes were allowed to come to room temperature, the suspensions were centrifuged, and the precipitates were washed with 5.0 ml of ether. The precipitates were finally dissolved in 0.5 ml of 97% formic acid, mixed with 5.0 ml of the counting fluid (9) and radioactivity was assayed in the liquid scintillation spectrometer.

Results and Discussion. A very significant reduction in the *in vivo* incorporation of labeled amino acids into the circulating serum albumin was observed in pantothenic acid-deficient rats (Fig. 1). It would appear, therefore, that the metabolism of serum proteins other than antibodies may be affected in this deficiency. Despite this reduction in the incorporation of labeled amino acids, the hepatic polysomal profiles of the pantothenic acid-deficient rats did not show any variation from the normal (Fig. 2). This observation suggests that the synthesis of messenger RNA in pantothenic acid deficiency is not impaired. The observation that the amount and distribution of the nascent peptides attached to the polysomes after intravenous injection of ^{14}C-amino acids were not altered

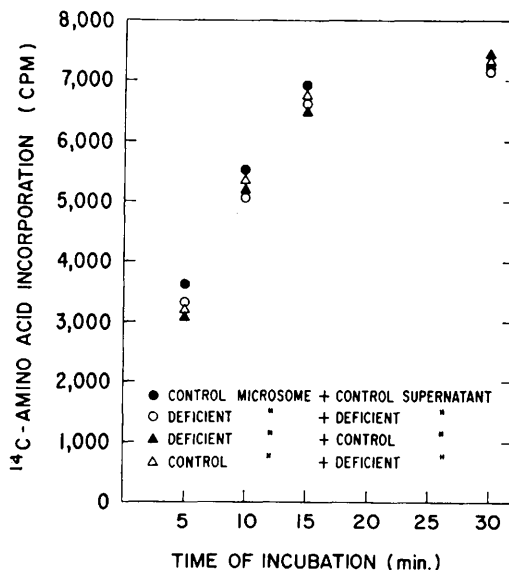

FIG. 4. Time course of ^{14}C-amino acid incorporation of the microsomes and supernatant fractions of 7 pantothenic acid-deficient and 7 control rat livers. Variations between animals of each group were not significant and only representative data are shown.

significantly in the pantothenic acid-deficient rats (Fig. 3) indicated that the translational efficiency of the polyribosomes was not impaired. Studies of ^{14}C-amino acid incorporation *in vitro* with the microsomal and supernatant fractions from control and pantothenic acid-deficient rats failed to demonstrate any functional defect in either the microsomes or the supernatant enzymes and cofactors of the pantothenic acid-deficient rat liver (Fig. 4). Since these data are based upon enzymatic activities of the same amount of microsomal protein, it is recognized that variations in liver size or in the content of liver microsomal protein between control and deficient animals might obscure real differences between the functional propensities of the two types of liver. However, we (Mahboob, Goldin, and Axelrod, unpublished observations) did not observe any significant effects of pantothenic acid deficiency upon the ratio of liver weight to body weight or upon the concentrations of the following per gram of liver: DNA, microsomal RNA, or microsomal protein. The decreased incorporation of amino acids into circulating albumin in pantothenic acid-deficient rats (Fig. 1), therefore, does not seem to be due to an alteration of the transcriptional and translational processes involved in protein synthesis by pantothenic acid-deficient rat hepatocytes (Figs. 2–4). Accordingly, we considered the possibility that the defect in pantothenic acid deficiency might be a cellular impairment of the ability to secrete newly synthesized proteins into the extracellular compartment. A preliminary study has given support to this suggestion. A defect in the intracellular transport of newly synthesized serum proteins in the liver of choline-deficient rats has been suggested by Oler and Lombardi (10).

Summary. The *in vivo* incorporation of ^{14}C-amino acids into circulating serum albumin was diminished in pantothenic acid-deficient rats. However, this deficiency induced no detectable changes in the liver polysomal profiles or the activities of the liver microsomal and soluble supernatant fractions in *in vitro* protein synthesis. In preliminary studies, indications were obtained that the intracellular transport of newly synthesized proteins was impaired in liver cells from pantothenic acid-deficient rats.

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