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Role of Vitamin B_{12} in Nucleic Acid Metabolism. V. Liver Deoxyriboaldolase Activity.* (22976)

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Vit. B_{12} deficiency has been associated with decreased nucleic acid content in rat liver (1,2), and microorganisms(3,4). This decreased nucleic acid content may be related to defective carbohydrate metabolism in view of reported reduction in ability of the deficient animal to utilize carbohydrate(5). Liver contains an enzyme system for synthesis of ribose and deoxyribose by condensation of C_2 and C_3 compounds(6,7). Lowered rate of formation of C_5 sugars, and hence of nucleic acids in liver, of vit. B_{12} deficient animals could, therefore, be due to lowered substrate concentrations of these C_2 and C_3 compounds, and/or to decreased activity of condensing enzymes to form the C_5 sugars. Accordingly, deoxyriboaldolase (DR-aldolase), the enzyme catalyzing the reaction: Glyceraldehyde-3-phosphate + acetaldehyde = Deoxyriboside-5-phosphate, was chosen for study.

Materials and methods. Vit. B_{12} deficient rats of Sprague-Dawley strain were raised as previously described(2). Livers were removed from exsanguinated animals with or without previous ether anesthesia, and 1 g portions were immediately homogenized in 10 ml 0.2 M Tris buffer (pH 7.4) in Potter-Elvehjem glass homogenizer. The 10% homogenate was centrifuged at room temperature for 10 minutes, and the centrifugate used as enzyme source. Livers from deficient and supplemented paired littermates were used for each enzyme experiment. In initial experiments, each incubation tube contained: 0.2 ml 0.1 M Fructose-1, 6-diphosphate, 0.2 ml 0.06 M Sodium-iodoacetate, 0.1 ml 10% (v/v) Acetaldehyde, Enzyme preparation (0.2-1.4 ml), 0.2 M Tris buffer, pH 7.4, total volume of mixture = 2 ml. Fructose 1,6-diphosphate solutions were prepared fresh from Barium salt (Mann) by adding equivalent amounts of Na_2SO_4 and removing resulting $BaSO_4$ precipitate by centrifugation. After 30 minutes incubation at 37°C, with tubes stoppered, 2 ml 10% trichloracetic acid was added. The mixture was rapidly stirred, centrifuged, and aliquots of supernatant assayed for deoxyribose by the colorimetric method of Dische: 2 ml acetic acid-H_2SO_4 reagent containing 1% diphenylamine was added to 1 ml sample. Color was developed by heating mixture for 10 minutes at 100°C. Optical densities at 610 mu and 650 mu were determined in Beckman DU spectrophotometer, and the Δ O. D. ($O.D._{610} - O.D._{650}$) compared to that of a standard DNA preparation. The findings are shown in Table I. The following modifications of above conditions for measuring enzyme activity were made in later experiments. An increased amount of sodium-iodoacetate (0.3 ml of solution not more than 6 weeks old) was used, and the solution of 10% acetaldehyde was made in 0.008M Tris buffer, pH 7.4. Better stoichiometry in the colorimetric assay was observed when 0.1 ml 10% acetaldehyde was added to every 30 ml acetic acid-H_2SO_4-diphenylamine reagent.

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TABLE I. Relative DR-aldolase Activity of Livers from Vit. B_{12}-Deficient Rats.

Age in wk	Sex	△ g body wt*	Relative activity†
9	♀	39	71– 87
11	♀	9	74– 93
12	♀	45	55–100
17	♂	57	73– 74
21	♀	26	68– 75
22	♀	39	73– 85
23	♀	21	74–100

* Difference in body wt between each pair of supplemented and deficient littermates.

† In each case, activity of liver of supplemented rat was arbitrarily taken as 100%. The values denote the range observed with 2 or 3 enzyme concentrations; this range was reduced to 10% or less in subsequent experiments with use of modified procedure in enzyme assay (Table II).

Color developed at room temperature (30-36°C) for 15-20 hours according to the method of Burton(8). The dichromatic method of colorimetric assay was again employed, using deoxyribose† as the standard. Optical densities were measured at 600 mu instead of 610 mu, and at 650 mu. After these alterations in incubation and colorimetric procedures were made, it was possible to obtain better stoichiometry when the enzyme preparation was present in concentrations ranging from 0.2 to 1.0 ml.

Results. Less than 10% variation in results between each enzyme concentration was obtained within this range. Table II summarizes the results obtained with this modified procedure for another series of vit. B_{12} deficient and supplemented animals. It can be concluded, from data presented above, that there is a definite decrease in enzymatic activity in livers of vit. B_{12} deficient rats, as compared to that in livers of supplemented littermates. Earlier studies showed that the per cent protein in liver from vit. B_{12} deficient rats is not altered(1). However, the nature of the crude enzyme system does not permit the conclusion that the DR-aldolase activity is indeed lower in the deficient animal. Since Fructose-1, 6-diphosphate is used as a source for triose-phosphate without exogenous aldolase added, the results may be interpreted as a change in either aldolase or DR-aldolase activity, or a combination of both. Further studies are needed to elucidate this point.

† California Foundation for Biochemical Research.

TABLE II. Apparent DR-aldolase Activity of Livers from Deficient (A1) and Supplemented (A3) Rats.

Diet, A	Body wt, g	Age, wk	Sex	μg DR formed/g liver*	Relative activity
1	204	36	♀	885	80
3	247			1110	100
1	230	36	♂	880	80
3	310			1197	100
1	250	37	♂	753	80
3	350			947	100
1	351	37	♂	803	73
3	459			1105	100
1	248	37	♀	662	68
3	284			977	100

* Avg of results from 3 enzyme concentrations.

Summary. An apparent decrease in liver deoxyriboaldolase activity was observed in livers from vit. B_{12} deficient rats as compared to the activity observed with livers from littermates supplemented with vit. B_{12}.

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