Effect of B-Vitamins on Biosynthesis of Ascorbic Acid from Glucose Cycloacetoacetate in Germinating Phaseolus Radiatus.* (25021)

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The authors(1) have shown that glucose occurs in combination with acetoacetate in germinating mung beans and that thiamine and pantothenic acid accelerate such biosynthesis. Reed(2) reported that co-carboxylase and CoA which contain thiamine and pantothenic acid respectively, are involved in metabolism of keto-acids and may thus help condensation of glucose with acetoacetate to form glucose cycloacetoacetate. Sure *et al.*(3) showed that deficiency of thiamine results in reduced storage of ascorbic acid in various tissues and endocrines, and Kennaway and Daff(4) reported that there is less ascorbic acid in the livers of mice kept on thiamine deficient diet. Smita *et al.*(5) have also observed that thiamine stimulates ascorbic acid biosynthesis in germinating mung beans and Thangamani and Sarma(6) showed that thiamine and pantothenic acid are also involved in greater biosynthesis of ascorbic acid from glucose cycloacetoacetate in germinating mung beans than with glucose alone. That reducing substance found during the process of germination of mung beans is ascorbic acid has recently been confirmed by the authors (7) through paper chromatographic technic.

Methods and Materials. Germination of mung beans was carried out as reported(1). Acetoacetate was added in the form of its sodium salt and in equimolar proportion with glucose. Glucose cycloacetoacetate (GCA) was prepared according to the method of West(8) as modified by Nath *et al.*(9). Due to its insolubility in cold water, it was dissolved in hot water and its solubility remained to the extent of 100 mg per 10 cc in cold condition. Pantothenic acid was used as Ca salt. After germination, the selected seeds were harvested and macerated with metaphosphoric-acetic acid mixture and their ascorbic acid contents estimated by the indophenol dye method(10). The results obtained after germination of the mung beans for different periods in presence of glucose and acetoacetate and glucose cycloacetoacetate with and without vitamins and their anti-vitamins are shown in Tables I and II.

Results. Both pyrithiamine and pantoyltaurine inhibit ascorbic acid synthesis in presence of acetoacetate and glucose (Table I), the inhibition being checked by the corresponding vitamins. Results with glucose cycloacetoacetate are represented in Table II.

Discussion. The role of thiamine and pantothenic acids as co-factors in bringing about

TABLE I. Effects of Pyrithiamine and Pantoyltaurine and Corresponding Vitamins on Biosynthesis of Ascorbic Acid from Glucose and Acetoacetate during Germination of Mung Beans.

	Fresh basis					
	mg/100 seedlings after			mg/100 g after		
Substances in germinating medium	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
Nil	1.15	2.05	2.25	9.2	10.25	11.25
Acetoacetate + glucose	1.54	3.82	4.14	15.4	24.83	24.84
Idem + pyrithiamine	1.4	3.3	4.01	14.0	21.4	24.06
" + " + thiamine	1.51	3.85	4.33	15.48	24.52	25.98
" + pantoyltaurine	1.3	3.11	3.65	13.0	20.21	21.9
" + " + pantothenate	1.85	3.97	4.69	18.5	25.8	28.14
" + thiamine + pantothenate	2.13	4.27	5.16	21.3	27.7	30.96

Concentrations: Acetoacetate (sodium salt), 70 mg/10 cc medium; glucose, 100 mg/10 cc; and 200 PPM each of pyrithiamine, pantoyltaurine, thiamine and calcium pantothenate.

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TABLE II. Effects of Pyrithiamine and Pantoyltaurine and Corresponding Vitamins on Biosynthesis of Ascorbic Acid from Glucose Cycloacetoacetate (GCA) during Germination of Mung Beans.

	Fresh basis					
	mg/100 seedlings after			mg/100 g after		
Substances in germinating medium	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
Nil	1.85	1.92	2.2	6.8	10.36	11.2
Glucose cycloacetoacetate (GCA)	1.46	2.8	2.1	11.48	16.8	24.6
Idem + pyrithiamine	1.36	2.23	3.51	10.88	13.38	21.06
" + " + thiamine	1.45	2.82	4.2	11.6	16.92	25.2
" + thiamine	1.53	3.25	4.4	12.24	26.0	27.65
" + pantoyltaurine	1.3	2.4	3.9	10.4	14.4	23.5
" + " + pantothenate	1.65	2.86	4.4	13.2	17.16	26.4
" + pantothenate	1.8	3.25	4.5	14.4	26.0	28.7

Concentrations: Glucose cycloacetoacetate, 100 mg/10 cc medium and 200 PPM each of pantoyltaurine, pyrithiamine, thiamine and calcium pantothenate.

enzymic biosynthesis of ascorbic acid from glucose cycloacetoacetate is indicated. These vitamins may be taking part both in condensation of glucose with acetoacetate to form GCA and in the further reaction leading to biosynthesis of ascorbic acid. In absence of these vitamins, the condensation reaction is hampered resulting in a low value of combined sugar, as reported previously, and consequently in lower output of ascorbic acid.

Summary. The anti-vitamins, *i.e.* pantoyltaurine and pyrithiamine, inhibited ascorbic acid biosynthesis from acetoacetate and glucose in germinating mung beans indicating a blocking of the condensation reaction of acetoacetate with glucose to form GCA. The corresponding vitamins reversed the inhibition by antivitamins. That pyrithiamine and pantoyltaurine inhibited synthesis of ascorbic acid in presence of GCA, and the corresponding vitamins reversed the inhibition. Cocarboxylase and CoA, of which thiamine and pantothenic acids are the constituents, are involved in both the condensation reaction of glucose with acetoacetate to form GCA and in the further metabolism of GCA to ascorbic acid.

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Ultrastructure and Site of Formation of Rabbit Papilloma Virus. (25022)

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Since its discovery in 1933(1), numerous attempts have been made to characterize physically the rabbit papilloma virus. The early conclusions from sedimentation, diffusion and viscosity measurements of purified virus protein preparation indicated an aspherical particle with a molecular weight of 47,100,000(2).

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