THE ROLE OF PANTOTHENIC ACID IN THE BIOSYNTHESIS OF ASCORBIC ACID IN THE RAT

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THE role of the vitamins of the 'B' group, in general, in the biosynthesis of ascorbic acid in the rat has been investigated by several workers. Thus Svirbely[1] found in 1936 that adequate amounts of vitamin 'B' factors are essential to obtain normal values for vitamin C in the organs of the rat. Guha and co-workers[2-4] have found that adequate thiamine, riboflavin, pantothenic acid and folic acid nutrition is a prerequisite to chloretone stimulation of ascorbic acid synthesis in the rat.

The work of Isherwood *et al.*[5] has shown that ascorbic acid is synthesised in the rat by the following pathway: D-Glucose → D-Glucuronic acid → L-Gulonic acid → L-ascorbic acid. King and his co-workers[6-8] have confirmed that glucose and glucuronic acid are precursors for the synthesis of ascorbic acid in the rat by tracer technique.

Sastry and Sarma[9] have found that thiamine is required for the conversion of glucuronic acid to ascorbic acid in the rat. Nath *et al.*[10] have shown that a condensation product of glucose and ethyl acetoacetate, glucose cyclo-acetoacetate, brings about an increased synthesis of ascorbic acid in germinating mung beans and also in rats. This was confirmed in our laboratory by radioactive tracer technique.[11] Further, it was shown that thiamine and pantothenic acid were required for the conversion of glucose cyclo-acetoacetate to ascorbic acid in germinating greengram.[11]

In the present investigation the role of pantothenic acid in the conversion of the precursors glucose cyclo-acetoacetate and glucuronic acid to ascorbic acid has been studied. Also, in view of the fact that D-glucuronolactone was found to be an intermediate in the conversion of D-glucose to L-ascorbic acid,[5-8] the effect of glucose-cyclo-acetoacetate on the urinary excretion of glucuronic acid has been studied.

Methods.—Pantothenic acid deficiency was produced in rats in the following manner: Ten weanling male albino rats, divided into two groups of five each, were housed in individual cages and fed control and pantothenic acid-deficient diets, recommended by Olson and Kaplan[12] for 8 weeks. After 6 weeks, when the rats kept on the deficient diet did not gain weight they were put in individual metabolism cages and were given the same diet as before. Urine collections were made for 48 hours in beakers containing 5 ml. of 10% oxalic acid solution and a little toluene. Ascorbic acid in the urine samples was estimated by the method of Roe and Kuether.[13] The rats were then given glucose-cyclo-acetoacetate (25 mg.) or D-glucurcnolactone (100 mg.) as the case may be, by intra-peritoneal injection of their aqueous solutions. The urinary excretion of ascorbic acid during the next 48 hours was determined in each case.

It was found desirable to estimate glucose-cyclo-acetoacetate in urine in order to see whether in pantothenic acid deficiency, this compound got accumulated. For this purpose, a highly sensitive spectrophotometric method was used. A solution of glucose-cyclo-acetoacetate in methanol was found to show maximum absorption at 250 mμ using Beckmann model DU quartz spectrophotometer.

Before estimating glucose-cyclo-acetoacetate in the urine samples, a preliminary purification of the samples was carried out, to eliminate interfering substances, in the following manner: The urine samples were repeatedly extracted with ethyl ether. The ether was evaporated and the residue was dissovled in 0·5 ml. of ethanol. The whole of this solution was applied as one spot on Whatman No. 1 filter-paper, along with a few spots containing pure glucose-cyclo-acetoacetate. The spots were subjected to ascending paper chromatography using the solvent mixture *n*-butanol, methanol, benzene and water in the ratio 2 : 1 : 1 : 1 as the mobile phase. The paper was then cut into vertical strips and the strips containing pure glucose-cyclo-acetoacetate were sprayed with 0·05% potassium permanganate solution. The spots were located by immediate decolourisation. The mean R_f value was determined and was found to be 0·83. From the strips containing the test samples, the regions corresponding to the R_f of the pure compound were cut off and eluted with methanol. The eluates were suitably diluted with methanol and their optical densities read out at 250 mμ.

Glucuronic acid excreted in the urine before and after an intraperitoneal injection of glucose-cyclo-acetoacetate was estimated by the method of Dische.[14] Normal rats, about 100 g. in weight, were used in this experiment.

Results and Discussion.—From Table I it may be seen that the normal excretion of ascorbic

acid was very much lowered in pantothenic acid deficiency. Also, whereas in the control rats the excretion of ascorbic acid increased after an injection of glucose-cyclo-acetoacetate, there was no such increase in the case of deficient rats, thereby showing a requirement of pantothenic acid for the conversion of glucose-cyclo-acetoacetate to ascorbic acid. Injection of D-glucurono lactone brought about an increase in ascorbic acid excretion both in control and in deficient rats, showing that pantothenic acid is not needed for the conversion of D-glucuronic acid to ascorbic acid.

TABLE I

Influence of pantothenic acid deficiency on (a) the synthesis of ascorbic acid in rat, from (1) glucose-cyclo-acetoacetate, and (2) D-glucuronolactone, (b) the metabolism of glucose-cyclo-acetoacetate

Rat group	Average urinary excretion of ascorbic acid (mg. per rat per day)					Average urinary excretion of glucose-cyclo-acetoacetate after an injection of 25 mg of the compound (mg. per rat per day)
	Before injection	After injection of glucose-cyclo-acetoacetate	Mean increase	After injection of glucurone	Mean increase	
Control	0·59	0·91	0·32	1·12	0·53	0·39
Deficient	0·34	0·38	0·04	0·88	0·54	1·023

TABLE II

Effect of an intraperitoneal injection of 25 mg. of glucose-cyclo-acetoacetate into rat on urinary excretion of glucuronic acid

Rat No.	Weight of the rat (gm.)	Amount of glucuronic acid excreted (mg. per rat per day)	
		Before injection	After injection
1	140	6·0	9·6
2	110	3·6	5·0
3	100	3·1	4·7
4	80	3·9	6·1

It may also be seen from the results presented in Table I that out of the injected amount of glucose-cyclo-acetoacetate, there was greater excretion of unmetabolised compound by the deficient rats than by the control rats indicating the need for pantothenic acid in the metabolism of glucose-cyclo-acetoacetate. It may further be pointed out that in both the groups, most of the injected compound was metabolised in the body of the rat.

The data in Table II shows that injection of glucose-cyclo-acetoacetate into rats brought about a significant increase in the urinary excretion of glucuronic acid. It has been shown earlier by other investigators that an increase in the urinary excretion of ascorbic acid is usually accompanied by an increase in the urinary excretion of glucuronic acid also.[15-16] It is possible that glucose-cyclo-acetoacetate may act as a precursor of ascorbic acid by first getting converted to glucuronic acid. In that case the reaction sequence can be written as follows :

$$\text{Glucose cyclo aceto acetate} \xrightarrow{(1)} \text{Glucuronic acid} + \text{Aceto acetate}$$

$$\text{Glucuronic acid} \xrightarrow{(2)} \text{Ascorbic acid}$$

The reaction (1) involves oxidation of the glucose part and splitting off of acetoacetate. For the latter, pantothenic acid in the form of coenzyme A may be necessary.

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