THE SITE OF INHIBITION OF ASCORBIC ACID SYNTHESIS BY LIVER PREPARATIONS FROM RATS DEPRIVED OF VITAMIN E

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Liver preparations from animals deprived of tocopherol synthesize ascorbic acid at a rate considerably lower than preparations from control animals (Caputto, McCay and Carpenter, 1958). Tocopherol, EDTA, and Co^{++}, Mn^{++}, Ce^{+++} salts independently reactivate the in vitro synthesis by preparations from E-deficient animals (McCay, Carpenter, Kitabchi and Caputto, 1959). A material which reacts with thiobarbituric acid (TBA) is produced by the preparations from the deficient animals and since the agents mentioned above which restored the normal production of ascorbate also stopped the production of TBA-reacting material, the hypothesis was advanced that both phenomena are related (Carpenter, Kitabchi, McCay and Caputto, 1959).

The enzymes in the liver preparations responsible for the conversion of glucuronic acid into ascorbic acid can be divided into the enzymes in the supernatant which catalyze the steps: D-glucuronic acid $\rightleftarrows$ L-gulonolactone and those in the microsomes which catalyze the reactions L-gulonolactone $\longrightarrow$ ascorbic acid (Kanfer, Burns and Ashwell, 1959).

The activity of the supernatant enzymes was determined with L-gulonolactone as substrate. A manometric method and a spectrophotometric procedure for determining TPN reduction were used. The latter method requires a preliminary dialysis which could eliminate possible inhibitors while the former method does not require such treatment. The interpretation of the results of manometric experiments with undialyzed preparations is hampered by the observation that

the increment of O_2 consumption due to the addition of substrate was higher in preparations from E-deficient animals, but on the other hand, the endogenous oxidation (not shown in the table) was considerably more in control preparations. Considering all of these factors, however, it appears that there is no inhibition of the enzymic steps carried out by the supernatant (Table I).

TABLE I

Reactions of the ascorbic acid synthesis sequence in preparations from livers of E-deficient rats and their controls

Dietary group	Enzyme preparation	Addition to test system	No. of exp.	μMole produced or consumed/hr./g. liver	TBA reaction [d]
				TPNH [a]	O.D. 535 mμ
E-deficient	dialyz. supernat.	---	8	3.74	0
E-sufficient	"	---	8	3.96	0
				Oxygen [b]	
E-deficient	supernatant	---	3	8.8	---
E-sufficient	"	---	3	3.6	---
				Ascorbate [c]	
E-deficient	microsomes	---	7	1.9	.484
	"	Tocopherol	2	3.3	.057
	"	Co	2	4.1	.016
	"	EDTA	2	4.1	.024
E-sufficient	"	---	7	3.8	.021
	"	Tocopherol	2	3.5	.018

[a] Determined spectrophotometrically at 340 mμ. [b] Determined manometrically. [c] Method of Roe and Kuether (1943). [d] Method of Ottolenghi (1959).

TESTS. For supernatant enzymes: a) spectrophotometric method: L-gulonolactone, 2.5 μmoles; TPN, 2 μmoles; $MgSO_4$, 1 μmole; dialyzed supernatant, 0.25ml; phosphate buffer pH 7.4, to complete 2 ml. b) manometric method: L-gulonolactone, 10 μmoles (side bulb); TPN, 1 μmole; diaphorase, 18 units; methylene blue, 0.02 μmole; supernatant, 0.3 ml.; phosphate buffer pH 7.4, to complete 1.3 ml.; 20% KOH, 0.2 ml. (in center well). For microsomal enzymes: L-gulonolactone, 2 μmoles; microsomes suspension, 0.25 ml., phosphate buffer, pH 7.4, to complete 1 ml. Values given are the mean differences between the incubation with and without L-gulonolactone, calculated for 1 hour at 37°C and 1 gram of tissue.

Results in Table I also show that there is a considerable decrease in the enzymic conversion of L-gulonolactone to ascorbic acid carried out by the

microsomes of E-deficient rats with respect to the control animals ($p < 0.001$ for the difference between 1.9 μmole and 3.8 μmole). This decrease, however, is not so marked as is the synthesis of ascorbic acid from glucuronic acid carried out by the original preparations (Caputto, McCay and Carpenter, 1958) from which the microsomes were obtained. This phenomenon is now being investigated, but there are already indications that it is related to the requirement for both supernatant and microsomes for maximum production of TBA-reacting material. The effects of those agents (tocopherol, Co^{++}, Mn^{++} , Ce^{+++} , and EDTA) which reactivate ascorbic acid synthesis from glucuronic acid, are also exerted on the reactions carried out by the microsomes.

A finding which may explain the unusually strong and early effect of the deprivation of vitamin E on the synthesis of ascorbic acid in vitro is that L-gulonolactone is either a substrate or a catalyst in the formation of TBA-reacting material.

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