INHIBITION OF SUCCINOXIDASE BY L-GULONOLACTONE OXIDASE IN LIVER PREPARATIONS FROM TOCOPHEROL-DEFICIENT RATS

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Despite the occurence of pronounced functional and structural tissue derangements in tocopherol deficiency, very few enzymes are known to be inhibited in this condition (Caputto, McCay and Carpenter, 1961). The possibility that this may be a consequence of the measuring procedures, which usually consist of singling out one activity, has been considered in this laboratory since it was found that the decrease of L-gulonolactone oxidase activity in tocopherol deficiency is not due to a decrease in the enzyme but to alterations in the medium (Kitabchi, et al, 1960; Caputto, Trucco and Kitabchi, 1961).

Succinoxidase was selected for this study because it has been shown, in various ways, to be concerned with tocopherol and lipid peroxidation (Ottolenghi, Bernheim and Wilbur, 1955; Tappel, Zalkin, 1959; Carton and Swingle, 1959; Edwin and Green, 1960). The present study confirms the observation of Corwin and Schwarz (1960) that succinoxidase activity deteriorates more rapidly in homogenates from tocopherol-deficient rats. Furthermore, it was seen that this effect could be prevented by the same factors which prevented the inhibition of gulonolactone oxidase in similar preparations (Kitabchi, et al, 1960). It was hypothesized that if both enzymes had a common mechanism of inhibition, the toxic effect that the activity of L-gulonolactone oxidase exerts on itself might be demonstrated in succinoxidase as well. Table I (Expts. 1a and 3a) reveals that the decline of succin-

TABLE I

Succinoxidase activity in liver preparations from tocopherol-sufficient and -deficient rats

Succinoxidase was determined manometrically. Ascorbic acid was determined by the 2,4-dinitro-phenyl-hydrazine method. Incubation system: liver preparation, 0.5 ml; NaCl, 300 μmoles; KCl, 12 μmoles, sodium phosphate buffer pH 7.4, 40 μmoles; $MgSO_4$, 4 μmoles; sodium succinate (in side arm) 60 μmoles; total volume 2.5 ml; gas phase, air; temp. 37°C. Values are corrected for endogenous respiration.

Exp. No.	Liver prep.†	Additions††	Incub. time (min.) 30	60	90	120	Ascorb. Acid synth.
			O_2 Consumed				
	Liver preparations from tocopherol-sufficient rats						
			μliters /30 min.				μmoles/ 120 min.
1 a	Homog.	-	74	94	69	74	
b	Mitoch.	-	103	69	62	52	
2 a	Mitoch.	-	78	88	67	76	
b	Mitoch.	GL, GL-oxidase	79	82	58	48	
c	Mitoch.	As 2 b; tocoph.	67	62	57	52	
3 a	Mitoch.	-	-	91	71	47	
b	Mitoch.	Ascorbate (20 μg)	-	90	64	52	
c	Mitoch.	Ascorbate (100 μg)	-	81	56	36	
	Liver preparations from tocopherol-deficient rats						
4 a	Homog.	-	93	56	31	22	
b	Mitoch.	-	109	88	81	56	
5 a	Mitoch.	-	54	80	73	74	0.0
b	Mitoch.	GL	95	79	70	65	0.05
c	Mitoch.	GL-oxidase	78	70	61	52	0.0
d	Mitoch.	GL, GL-oxidase	98	53	16	13	0.20
e	Mitoch.	Ascorbate (100 μg)	31	00	03	07	
f	Mitoch.	Ascorbate (100 μg) + Tocoph. (100 μg)	94	30	44	37	
6 a	Mitoch.	-	86	88	90	78	
b	Mitoch.	"Tocoph-deficient" microsomes	103	117	70	57	
c	Mitoch.	As 5 b; GL	133	85	29	13	0.28
7 a	Mitoch.	-	115	115	91	70	
b	Mitoch.	GL; GL-oxidase	86	17	06	02	
c	Mitoch.	As 7 b; 50 μg tocoph.	126	76	38	37	
d	Mitoch.	As 7 b; 100 μg tocoph.	122	77	50	23	
e	Mitoch.	As 7 b; 73 μg EDTA	105	98	70	56	
f	Mitoch.	As 7 b; 237 μg $MnCl_2$	97	95	61	61	
8 a	Mitoch.	GL, GL-oxidase	132	56	01	07	0.25
b	Mitoch.	1.5 μg malonaldehyd	98	104	75	84	

oxidase activity was greater in homogenates from tocopherol-deficient animals than in homogenates from controls animals, but no difference was found when the activities of the isolated mitochondria obtained from the respective homogenates were compared (Table I, expts. 1b and 3b). This feature is in common with what had been observed in the inhibition of L-gulonolactone which was also conditioned by the medium in which the reaction was carried out (Kitabchi, et al, 1960).

The addition of the soluble L-gulonolactone oxidase system (enzyme plus substrate, expts. 4d, 6b, 7a) to the mitochondrial succinoxidase from tocopherol-deficient rats invariably results in an accelerated decay of the latter activity which usually became minimal after 60 minutes. The separate addition of either L-gulonolactone oxidase or its substrate had no effect on succinoxidase and in no case did the L-gulonolactone oxidase activity have a significant effect on liver mitochondria obtained from tocopherol-sufficient rats (Expt. 2b). All of the experiments mentioned in the foregoing were carried out with soluble preparations of L-gulonolactone oxidase. In order to examine these apparently regulatory mechanisms under conditions more closely approximating those in the cell, it became of interest to examine the effect of particulate L-gulonolactone oxidase in the microsomes. Experiments 5a, b and c show that the activity of this enzyme in the microsomes obtained from tocopherol-deficient rats was as inhibitory as the soluble preparations of L-gulonolactone oxidase. These experiments were carried out with quantities of mitochondria and microsomes

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†Homogenate: 10 per cent suspension of liver in 0.25 sucrose solution; Mitochondria: suspension of twice-washed mitochondria in one-half of the original homogenate volume of either 0.25 M sucrose (Expts. 1, 2, 3, and 6), or 0.15 M sodium phosphate buffer (Expts. 4, 5 and 7).

††GL refers to 24 μmoles of L-gulonolactone (added to the side arm); GL-oxidase refers to 0.2 ml of L-gulonolactone oxidase prepared as described previously (Kitabchi, McCay, Carpenter, Trucco and Caputto, 1960) under the designation "40 fraction". D-α-tocopherol was added as a 1 per cent solution in alcohol.

obtained from equal weights of liver, which emphasizes the importance of this phenomenon as part of the regulatory mechanism of the cell.

The reaction product of L-gulonolactone oxidase (ascorbic acid) appears to be mediator in the inhibitory sequence (Expt. 5e). Its inhibitory effect is manifested in preparations from tocopherol-deficient animals and the effect is substancially reduced by the addition of tocopherol *in vitro* (Expt. 5f). The addition of ascorbic acid *in vitro* has no effect on the mitochondrial succinoxidase of tocopherol-sufficient rats (Expt. 3). However, as pointed out previously in an earlier report on the autoinhibition of L-gulonolactone oxidase activity (Caputto, Trucco and Kitabchi, 1961), it cannot be stated positively whether or not ascorbic acid is the only initiator of the inhibitory sequence. In addition to tocopherol, the addition of EDTA or Mn^{++} salts substancially prevent the accelerated decline of succinoxidase activity induced by the gulonolactone oxidase system (Expt. 7). This again implicates the process of lipid peroxidation as another step in the inhibitory sequence and, consequently, malonaldehyde was studied as a possible intermediate responsible for the inhibition but was found to have no effect (Expts. 8a and b).

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