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The vitamin E activity of 5 different tocopheramines on muscular dystrophy in chicks

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With 1 figure and 4 tables

(Received January 24, 1970)

Vitamin E activity of some tocopheramines and their monomethyl-derivatives has been reported by several investigators. Thus, FARBER et al. (1953) (1) found α-tocopheramine to be active against muscular dystrophy in vitamin E deficient rabbits. Using the dialuric acid hemolysis test, SCHWIETER et al. (1966)[1]) (2) found α-, β-, γ- and δ-tocopheramines to have biological activity similar to the corresponding tocopherols in rats. N-methylation was found to reduce the activity of α-tocopheramine and to increase the activity of β- and γ-tocopheramine. N-dimethylation caused inactivation in all cases. GLOOR et al. (1966)[1]) (3) found that α-tocopheramine and N-methyl-γ-tocopheramine which were highly active, were not transformed to α-tocopherol in the body but were degraded to the corresponding „Simon metabolites". In the rat reproduction assay, BIERI and MASON (1968)[1]) (4) found N-methyl-β-tocopheramine to have a potency of about 89 per cent of that of α-tocopherol. Against exudative diathesis in chicks, BIERI and PRIVAL (1967)[1]) (5) found α-tocopheramine N-methyl-β-tocopheramine and N-methyl-γ-tocopheramine to have approximately the same preventive effect as α-tocopherol whereas β- and γ-tocopheramine had less activity. Further, α-tocopheramine and N-methyl-β-tocopheramine had approximately the same preventive effects against encephalomalacia in chicks as α-tocopherol had whereas the activity of β-tocopheramine was less.

In the present study we have examined the effect of all-rac α-tocopheramine, all-rac-N-methyl-γ-tocopheramin, all-rac-N, N-dimethyl-γ-tocopheramine, all-rac-δ-tocopheramine and all-rac-5,7-dimethyl-tocamine on muscular dystrophy (white striation of skeletal muscles) in chicks reared on a vitamin E deficient diet.

Experimental

The chicks were crossbreed of New Hampshire and White Leghorn received day-old from a local dealer. They were given the vitamin E deficient starter ration indicated in reference (6) for 5–7 days. Thereafter they were divided into groups of 10 and given the vitamin E deficient basal diet with low content of casein indicated in reference (7). Some of the groups received the basal diet without supplement, whereas other groups received the diet supplemented with graded levels of all-rac-α-tocopheryl acetate or graded levels of the tocopheramines to be tested. The ratio between two consecutive levels was in all cases 1.39. The experimental feeding lasted 5 weeks.

Four experiments were carried out.

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[1]) The tocopheramines and tocopherol(s) referred to by these authors were the d, l-forms, (all-rac-forms).

In experiments 1 and 2 the effects of all-rac-α-tocopheramine and all-rac-N-methyl-γ-tocopheramine were compared with the effect of all-rac-α-tocopheryl acetate.

In experiment 3 the effects of all-rac-N,N-dimethyl-γ-tocopheramine were compared with the effect of all-rac-α-tocopheryl acetate.

In experiment 4 the effects of all-rac-δ-tocopheramine, all-rac-N,N-dimethyl-γ-tocopheramine and all-rac-5,7-dimethyl-tocamine were compared with the effect of all-rac-α-tocopheryl acetate, but only in one dose.

At autopsy after 5 weeks of feeding, the degree of striation of breast muscles was scored as follows: zero = no striation, 1 = slight striation, 2 = moderate striation, 3 = marked striation.

Results and discussion

The results are presented in tables 1, 2 and 3 and in fig. 1.

From experiments 1 and 2 (table 1) it is seen that at each dose level the degree of white striation is not much different for all-rac-α-tocopheryl acetate, all-rac-α-tocopheramine and all-rac-N-methyl-γ-tocopheramine.

From experiment 3 (table 2) is seen that at each of the dose levels tested the degree of white striation is somewhat more marked with all-rac-N, N dimethyl-γ-tocopheramine than with all-rac-α-tocopheryl acetate. With all-rac-5,7-dimethyl-tocamine, the degree of white striation was about the same as that with all-rac-α-tocopheryl acetate given at dose levels of one fifteenth of those in which all-rac-5,7-dimethyl-tocamine was tested.

From experiment 4 (table 2) it appears that at the 10 mg % level the degree of striation was slight with all-rac-5,7-dimethyl-tocamine, moderate to marked with all-rac-δ-tocopheramine, and zero with all-rac-α-tocopheryl acetate and all-rac-N, N-dimethyl-γ-tocopheramine.

In table 3 the results of the four experiments are summarized in the following way:

For each dose level of a given compound the incidence of striation is calculated per 10 chicks and multiplied by the degree (score) of the striation. The figure calculated in this way is then divided by the corresponding figure calculated from the group receiving the unsupplemented basal diet in the same experiment, and multiplied by 100. In the cases where a given compound is tested at the same dose level in more than one experiment the values presented are mean values from all the pertinent experiments.

In fig. 1 the figures from table 3 are plotted linearly against the dosage of the compounds administered (logarithmically). In this way each of the compounds tested are represented by a curve which has the from of a straight line the range between 15 and 85 on the vertical axis. Therefrom the activities of one mg of the different tocopheramines can be calculated in terms of the activity of one mg all-rac-α-tocopheryl acetat, and further converted to activity of one mol of tocopheramine in terms of the activity of one mol all-rac-α-tocopheryl-acetate or one mol all-rac-α-tocopheryl.

The molar activities of the different tocopheramines tested by us against muscular dystrophy in chicks are presented in table 4 together with the molar activities of different tocopheramines tested by other investigators against other signs of vitamin E deficiency. The activity of one mol all-rac-α-tocopheryl acetat and one mol all-rac-α-tocopherol are arbitrarily taken as 100.

In most cases the activities found by us do not differ greatly from the activities reported by other workers. The most striking difference concerns all-rac-N,N-dimethyl-γ-tocopheramine. The activity of this compound in the hemolysis test was

Table 1

mg chroman-derivative per 100 g diet	Experiment 1.			Experiment 2.		
	Incidence and mean score of severity of striation of breast muscles.[2])					
	all-rac-α-tocopheryl acetate	all-rac-α-tocopheramin	all-rac-N-methyl-γ-tocopheramin	all-rac-α-tocopheryl acetate	all-rac-α-tocopheramin	all-rac-N-methyl-γ-tocopheramin
0.373			10^{10} (2.10)			
0.519	10^{10} (2.15)	10^{10} (2.00)	10^{10} (2.10)			
0.721	8^{8} (2.00)	10^{10} (2.35)	10^{10} (2,25)	9^{10} (1.30)	9^{10} (1.13)	8^{10} (1.70)
1.000	9^{10} (1.85)	8^{10} (1.55)	9^{9} (1.94)	9^{10} (1.15)	6^{10} (0.65)	8^{10} (1.08)
1.39	8^{10} (0.93)	9^{10} (1.70)	8^{9} (1.17)	3^{10} (0.15)	2^{10} (0.08)	6^{10} (0.90)
1.93	5^{9} (0.72)	4^{10} (0.70)	5^{10} (0.48)	2^{10} (0.25)	3^{10} (0.25)	2^{10} (0.10)
2.69	0^{10} (0.00)	0^{10} (0.00)		0^{10} (0.00)	0^{10} (0.00)	1^{10} (0.10)
3.73				0^{10} (0.00)	0^{10} (0.00)	0^{10} (0.00)
	Control[1])			Control[1])		
	8^{8} (2.25)			16^{18} (2.06)		

[1]) Group without added chroman-derivative

[2]) Elevated figures indicate number of chicks in group; figures in parenthesis indicate mean score of severity.

Table 2.

mg chroman-derivative per 100 g diet	Experiment 3.			Experiment 4.			
	Incidence and mean score of severity of striation of breast muscles.[2])						
	all-rac-α-tocopheryl acetate	all-rac-N, N-dimethyl-γ-tocopheramin	all-rac-5, 7-dimethyl-tocamin	all-rac-α-tocopheryl-acetate	all-rac-δ-tocopheramin	all-rac-N, N-dimethyl-γ-tocopheramin	all-rac-5, 7-dimethyl-tocamin
0.519	9^{10} (1.85)	10^{10} (2.20)					
0.721	9^{10} (1.75)	10^{10} (1.80)					
1.00	8^{9} (1.17)	10^{10} (1.75)					
1.39	4^{10} (0.50)	8^{10} (1.40)					
1.93	1^{10} (0.15)	4^{10} (0.45)					
2.69	0^{10} (0.00)	4^{10} (0.40)					
3.73			10^{10} (2.10)				
5.19			9^{10} (1.85)				
7.21			7^{10} (1.30)				
10.0			7^{10} (0.95)	0^{10} (0.00)	10^{10} (2.55)	0^{10} (0.00)	9^{10} (1.00)
13.9			5^{10} (0.48)				
19.3			2^{10} (0.15)				
	Control[1])			Control[1])			
	20^{20} (2.23)			10^{10} (2.75)			

[1]) Group without added chroman-derivative

[2]) Elevated figures indicate number of chicks in group, figures in parenthesis indicate mean score of severity.

Table 3. Summary of experiments 1–4

mg chroman-derivative per 100 g diet	Mean values of incidence x score of striation in the experimental groups as per cent of mean values x score of striation in the unprotected control group.					
	○ all-rac-α-tocopherol acetate	● all-rac-α-tocopheramin	⊙ all-rac-N-methyl-γ-tocopheramin	× all-rac-N, N-dimethyl-γ-tocopheramin	■ all-rac-δ-tocopheramin	□ all-rac-5, 7-dimethyl-tocamin
0.373			93.3			
0.519	85.2	88.9	93.3	98.6		
0.721	74.5	80.0	88.2	80.7		
1.00	57.7	38.2	66.7	78.5		
1.39	14.9	39.1	35.6	50.2		
1.93	6.5	8.4	5.9	8.1		
2.69	0	0	0.5	7.1		
3.73	0	0	0			76.3
5.19						60.5
7.21						33.1
10.00	0			0	92.7	28.4
13.9						8.7
19.3						1.0

Table 4. Comparative biopotencies of tocopheramines and α-tocopherol.[1])

Compound	Test method and species.					
	Rats		Chicks			Rabbits
	Resorption gestation	Hemolysis test[3])	Encephalomalacia[4])	Exudative diathesis[4])	Muscular dystrophy[5])	Muscular dystrophy[6])
all-rac-α-tocopherol	100		100	100		100
all-rac-α-tocopheryl acetate	100	100			100	
all-rac-α-tocopheramine	100[2])	87	98	111	100	< 25
all-rac-N-methyl-α-tocopheramine		< 10				
all-rac-N, N-dimethyl-α-tocopheramine		< 10				
all-rac-β-tocopheramine		26	18	48		
all-rac-N-methyl-β-tocopheramine	89[7])	132	110	101		
all-rac-γ-tocopheramine		20		37		
all-rac-N-methyl-γ-tocopheramine	90[8])	125		89	87	
all-rac-N, N-dimethyl-γ-tocopheramine		< 4			72	
all-rac-δ-tocopheramine		[9])			< 5	
all-rac-N-methyl-δ-tocopheramine		[9])				
all-rac-N, N-dimethyl-δ-tocopheramine		[9])				
all-rac-5, 7-dimethyltocamine		[9])			18	

[1]) Calculated on molar basis.
[2]) Smith et al. (1942). (8)
[3]) Schwieter et al. (1966). (2)
[4]) Calculated from Bieri and Prival (1967). (5)
[5]) Present study.
[6]) Calcuted from Farber et al. (1953). (1)
[7]) Calcuted from Bieri and Mason (1968). (4)
[8]) U. Gloor cited by Bieri and Mason (1968). (4)
[9]) Activity less than 80 % of all-rac-α-tocopherol.

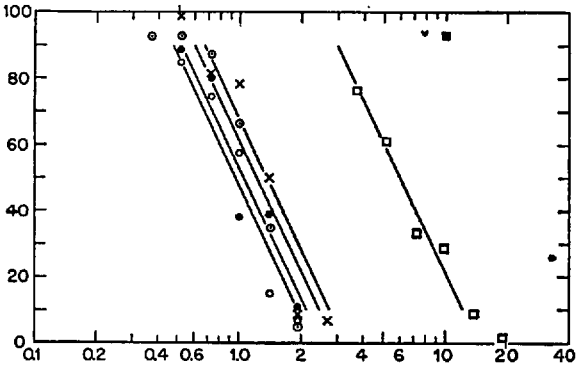

Fig. 1. Horizontal: mg chroman derivative per 100 g diet, logarithmic scale. Vertical: mean values of incidence x score of striation in the experimental groups as per cent of incidence x score of striation in the unprotected control group (table 3), linear scale.

- ○ all-rac-α-tocopheryl acetate.
- ● all-rac-α-tocopheramine
- ⊙ all-rac-N-methyl-γ-tocopheramine
- × all-rac-N, N-dimethyl-γ-tocopheramine
- □ all-rac-5, 7-dimethyl-tocamine
- ■ all-rac- -tocopheramine.

found by SCHWIETER et al. (1966) (2) to be less than 4 whereas we found an activity of 72 against muscular dystrophy in chicks. It must be born in mind, however, that in the tests carried out by Schwieter et al. the compounds were given as one single dose whereas in our tests the compounds were mixed with the diet and given daily. According to BIERI (1969) (9) the effects of methylated tocopheramines presuppose demethylation *in vivo*. Demethylation *in vivo* of N, N-dimethyl-γ-tocopheramine and of N-methyl-γ-tocopheramine was demonstrated by BIERI (1969) (9), and further, N, N-dimethyl-γ-tocopheramine was found to be stored in the liver to the same extent as the monomethyl derivative. It is possible therefore, that one single dose of the dimethyl-compound cannot provide a sufficient level of the active demethylated compound in the tissue, whereas continuous dosage can.

Acknowledgement

Wir danken F. Hoffmann-La Roche & Co. A. G. Basel, Schweiz, für die freundliche Überlassung der in dieser Arbeit benutzten Tocopheramine.

Summary

The activities of all-rac-α-tocopheryl acetate and 5 different tocopheramines against muscular dystrophy in vitamin E deficient chicks were determined.

The molar activities of the tocopheramines as per cent of the molar activity of all-rac-α-tocopheryl acetate were as follows:

All-rac-α-tocopheramine	100
all-rac-N-methyl-γ-tocopheramine	87
all-rac-N, N-dimethyl-γ-tocopheramine	72
all-rac-δ-tocopheramine	< 5
all-rac-5, 7-dimethyl-tocamine	18

Zusammenfassung

Die Aktivitäten von all-rac-α-Tocopherylacetat und 5 verschiedenen Tocopheraminen gegen Muskel-Dystrophie in Vitamin-E-defizienten Küken wurden bestimmt.

Die molären Aktivitäten der Tocopheramine als Procent der molären Aktivität des all-rac-α-Tocopherylacetats waren wie unten angegeben:

all-rac-α-Tocopheramin	100
all-rac-N-methyl-γ-Tocopheramin	87
all-rac-N, N-dimethyl-Tocopheramin	72
all-rac-δ-Tocopheramin	< 5
all-rac-5, 7-dimethyl-Tocamin	18

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