INFLUENCE OF DIETARY ASCORBIC ACID, NORDIHYDROGUAIARETIC ACID AND CYSTINE ON VITAMIN E DEFICIENCY SYMPTOMS IN CHICKS

by

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Feeding of highly unsaturated fatty acids to chicks in the absence of vitamin E causes the previously described symptoms: exudative diathesis and encephalomalacia. The frequency and severity of these symptoms can be modified by variation of certain components of the diet such as fat, protein, and salts.

The present studies deal with the effect of substances which may protect vitamin E in the body or may influence the utilization of proteins.

TABLE I

BASAL DIETS

	Basal diet no. 97[1]	Basal diet no. 122[2]	Basal diet no. 124[3]
Casein, unextracted	150 g	—	230 g
Milk powder[4]	—	—	180 g
Dried yeast, unextracted	—	—	50 g
Dried yeast, ether-extracted	100 g	—	—
Hog liver, dried	—	350 g	—
Potato starch	—	610 g	250 g
Sucrose	449 g	—	—
Gelatine	80 g	—	—
Gum arabic	50 g	—	—
Salt mixture[5]	50 g	40 g	40 g
Sodium chloride	20 g	—	—
Choline chloride	1 g	—	—
Lard	—	—	250 g
Cod liver oil	100 g	—	—
Vitamin K substitute[6]	10 mg	5 mg	5 mg
Vitamin A, D mixture[7]	—	—	1 drop 6 × weekly
Vitamin D mixture[8]	—	1 drop 6 × weekly	—

[1] Strongly exudate producing.
[2] Strongly encephalomalacia producing.
[3] Strongly encephalomalacia producing.
[4] Containing 6% fat as determined by GERBER's method.
[5] McCOLLUM's Salt mixture no. 185 supplemented with 13.5 mg KI, 139 mg $CuSO_4 \cdot 5H_2O$, 556 mg $MnSO_4 \cdot 4H_2O$ per 100 g.
[6] Tetra-sodium-salt of 2-methyl-1.4-naphthohydroquinone-diphosphoric acid ("Synkayvite, Roche").
[7] Vitamin A concentrate from fish liver oil (containing 10^6 units per g) 0.300 g, vitamin D concentrate (Delsterol, containing 200 000 chick units per g) 0.200 g, oleic acid 24.500 g. The concentrates were obtained from Distillations Products, Inc., Rochester, N. Y. 1 drop (29 mg) represents 350 units A and 46 chick units D.
[8] The same as [7] but without vitamin A.

References p. 513.

Preliminary trials with ascorbic acid and lemon juice were reported in a paper by DAM AND GLAVIND[1]. They showed that a certain amount of ascorbic acid delayed the development of the exudates somewhat without preventing them. We therefore undertook a study of the influence of larger amounts of the natural synergist of the anti-oxydant vitamin E, *viz.*, ascorbic acid, the unnatural synergist nordihydroguaiaretic acid, and the protein sparing amino-acid cystine.

The technique was that described by DAM[2].

The diets were modifications of the basal diets listed in Table I.

RESULTS AND CONCLUSIONS

The results of the feeding experiments are listed below.

The following abbreviations are used: d = dead, k = killed.

The number of days from the beginning of the feeding until the appearance of symptoms are indicated in columns 3 and 5 for exudates and encephalomalacia respectively.

GROUP 97

Basal Diet 97 without addition. Started Nov. 11, 1947

Chick no.	Exudate	Days	Encephalomalacia	Days	Duration of experiment, days	Weight at start	Weight at end
5833	+	27	o		27 k	40	93
5834	+	17	o		27 k	40	138
5835	+	13	o		14 k	40	95
5836	o		o		27 k	44	115
5837	+	17	o		27 k	40	142
5838	o		o		27 k	38	141
5839	+	17	o		27 k	36	144
5840	+	18	o		27 k	28	98
5841	+	13	o		14 k	40	103
5842	o		o		27 k	40	124
5843	o		o		27 k	40	129
5844	+	27	o		27 k	40	156

GROUP 98

Basal Diet 97 + 0.5 % cystine. Started Nov. 11, 1947

Chick no.	Exudate	Days	Encephalomalacia	Days	Duration of experiment, days	Weight at start	Weight at end
5845	+	21	o		27 d	40	114
5846	+	17	o		27 d	30	110
5847	o		o		6 d	34	30
5848	o		o		4 d	34	25
5849	o		o		27 k	34	134
5850	o		o		18 d	35	110
5851	+	17	+	17	17 k	40	114
5852	o		o		27 k	40	220
5853	o		o		27 k	38	169
5854	o		+	27	27 k	38	127
5855	o		o		15 d	38	100
5856	o		o		27 k	40	230

References p. 513.

GROUP 100

Basal Diet 97 + 0.05 % cystine. Started Nov. 11, 1947

Chick no.	Exudate	Days	Encephalomalacia	Days	Duration of experiment, days	Weight at	
						start	end
5869	+	17	o		20 k	40	100
5870	+	16	o		20 k	38	90
5871	+	20	o		27 k	40	148
5872	+	15	o		24 d	32	102
5873	+	17	o		20 k	34	80
5874	+	16	o		27 k	40	101
5875	+	16	o		17 d	40	100
5876	+	14	o		15 d	36	79
5877	+	27	o		27 d	30	70
5878	?	18	o		27 k	39	127
5879	o		o		10 d	34	39
5880	+	18	o		27 k	34	153
5881	o		o		27 k	34	119
5882	o		o		27 k	35	144

GROUP 101

Basal diet 97 without addition. Started Nov. 25, 1947

Chick no.	Exudate	Days	Encephalomalacia	Days	Duration of experiment, days	Weight at	
						start	end
5883	+	24	o		27 d	35	60
5884	+	24	o		27 d	38	80
5885	o		o		22 d	40	50
5886	+	24	o		29 k	40	105
5887	+	20	o		27 d	32	72
5888	o		o		13 d	40	34
5889	o		o		29 d	40	83
5890	+	18	o		24 d	33	80
5891	+	20	o		23 d	30	95

GROUP 102

Basal diet 97 + 0.5 % ascorbic acid. Started Nov. 25, 1947

Chick no.	Exudate	Days	Encephalomalacia	Days	Duration of experiment, days	Weight at	
						start	end
5892	+	27	o		56 k	39	273
5893	o		o		4 d	30	25
5894	o		o		56 k	32	170
5895	o		o		56 k	39	320
5896	o		o		56 k	38	250
5897	+	25	o		56 k	34	290
5898	o		o		56 k	36	250
5899	o		o		56 k	37	260
5900	o		o		56 k	30	340

References p. 513.

GROUP 106

Basal diet 97 without addition. Started Dec. 17, 1947

Chick no.	Exudate	Days	Encephalomalacia	Days	Duration of experiment, days	Weight at start	Weight at end
5918	+	12	o		35 d	30	71
5919	o		o		36 d	30	89
5920	o		o		21 d	29	50
5921	+	18	o		42 d	33	137
5922	o		o		47 k	30	120
5923	o		o		17 d	26	70
5924	+	18	+	19	19 k	28	65
5925	o		+	16	16 k	29	64
5926	+	18	o		47 k	29	160
5927	o		o		28 d	28	50

GROUP 107

Basal diet 97 + 0.05 % cystine. Started Dec. 17, 1947

Chick no.	Exudate	Days	Encephalomalacia	Days	Duration of experiment, days	Weight of start	Weight of end
5928	+	33	o		33 d	26	112
5930	+	14	o		46 d	31	155
5931	o		o		47 k	29	172
5933	+	14	o		47 k	32	190
5937	+	26	o		28 d	35	110

GROUP 108

Basal diet 97 + 0.1 % cystine. Started Dec. 17, 1947

Chick no.	Exudate	Days	Encephalomalacia	Days	Duration of experiment, days	Weight at start	Weight at end
5938	o		o		47 k	29	163
5939	+	24	o		28 d	30	74
5940	+	18	o		47 k	30	258
5942	o		o		31 d	30	118
5944	o		+	16	16 d	36	85
5945	o		o		47 k	28	210
5946	+	18	o		47 k	31	215
5947	+	24	o		47 k	29	180

GROUP 109

Basal diet 97 + 0.5 % cystine. Started Dec. 17, 1947

Chick no.	Exudate	Days	Encephalomalacia	Days	Duration of experiment, days	Weight at start	Weight at end
5948	o		o		33 d	31	132
5949	o		o		47 k	30	326
5952	o		+	35	37 d	27	140
5953	o		o		47 k	28	328
5954	o		o		46 d	30	267
5956	+	47	o		47 k	30	332
5957	o		o		21 d	32	50

References p. 513.

GROUP 110

Basal diet 97 + 1 % cystine. Started Dec. 17, 1947

Chick no.	Exudate	Days	Encephalomalacia	Days	Duration of experiment, days	Weight at	
						start	end
5958	o		o		21 d	33	78
5959	o		o		39 k	34	358
5960	+	26	+	30	30 k	38	180
5961	o		+	24	24 k	32	132
5962	o		o		39 k	30	146
5964	o		o		21 d	31	90
5966	o		o		39 k	30	200
5967	o		o		25 d	30	68

GROUP 112

Basal diet 97 + 0.5 % ascorbic acid. Started Dec. 17, 1947

Chick no.	Exudate	Days	Encephalomalacia	Days	Duration of experiment, days	Weight at	
						start	end
5979	o		o		47 k	34	250
5980	o		o		47 k	33	278
5981	o		o		28 d	32	65
5982	o		o		43 d	32	220
5983	o		o		30 d	31	100
5984	o		o		32 d	32	200
5985	o		o		47 k	32	294
5986	o		o		47 k	28	238
5987	o		o		47 k	35	252

GROUP 122

Basal diet 122 without addition. Started Jan. 14, 1948

Chick no.	Exudate	Days	Encephalomalacia	Days	Duration of experiment, days	Weight at	
						start	end
6083	o		+	19	24 d	44	160
6084	o		o		20 d	38	100
6085	o		+	33	37 d	32	152
6086	o		+	29	37 d	34	145
6087	o		+	35	43 k	48	174
6088	o		+	21	27 k	38	120
6089	o		+	15	35 d	42	170
6090	o		+	17	17 d	34	79
6091	o		+	21	21 k	34	86
6092	o		o		8 d	33	50

References p. 513.

GROUP 123

Basal diet 122 + 0.5 % ascorbic acid. Started Jan. 14, 1948

Chick no.	Exudate	Days	Encephalomalacia	Days	Duration of experiment, days	Weight at	
						start	end
6093	o		o		6 d	34	41
6094	o		+	42	43 k	41	202
6095	o		+	36	36 d	36	90
6096	o		+	27	34 d	43	162
6097	o		o		34 d	40	140
6098	o		+	21	23 d	33	92
6099	o		o		7 d	44	52
6100	o		+	31	34 d	34	136
6101	o		+	23	23 k	36	94
6102	o		o		43 k	36	120

GROUP 124

Basal diet 124 without addition. Started Jan. 14, 1948

Chick no.	Exudate	Days	Encephalomalacia	Days	Duration of experiment, days	Weight at	
						start	end
6103	o		+	19	19 k	44	70
6104	o		+	35	35 d	39	102
6105	o		+	23	23 k	41	100
6106	o		+	20	20 k	42	100
6107	o		+	19	19 k	40	74
6108	o		+	21	21 k	38	110
6109	o		+	43	43 k	43	132
6110	o		+	37	37 k	41	66
6111	o		+	26	20 k	44	80
6112	o		+	20	26 d	38	112

GROUP 125

Basal diet 124 + 0.5 % ascorbic acid. Started Jan. 14, 1948

Chick no.	Exudate	Days	Encephalomalacia	Days	Duration of experiment, days	Weight at	
						start	end
6113	o		o		43 k	30	290
6114	o		o		43 k	32	309
6115	o		o		4 d	29	40
6116	o		o		43 k	40	178
6117	o		o		43 k	42	262
6118	o		o		43 k	45	296
6119	o		o		43 k	34	362
6120	o		+	31	31 k	38	146
6121	o		o		43 k	41	276
6122	o		o		18 d	38	67

References p. 513.

GROUP 132

Basal diet 97 + 0.05 % cystine. Started Feb. 3, 1948

Chick no.	Exudate	Days	Encephalomalacia	Days	Duration of experiment, days	Weight at start	Weight at end
6184	+	29	o		31 d	40	106
6185	+	30	o		37 k	46	180
6186	+	35	o		37 d	44	130
6187	+	27	o		34 d	40	138
6188	o		o		25 d	36	84
6189	+	37	o		37 k	44	160
6190	+	20	o		36 d	46	130
6191	+	20	o		32 k	40	140
6192	+	20	o		36 d	44	180
6193	+	30	o		30 d	41	86
6194	+	28	o		34 d	38	100
6195	o		o		19 d	40	58
6196	+	34	o		37 k	41	162
6197	o		o		37 k	38	120
6198	o		o		32 d	38	84

GROUP 134

Basal diet 97 + 0.05 % cystine + 0.5 % ascorbic acid. Started Feb. 3, 1948

Chick no.	Exudate	Days	Encephalomalacia	Days	Duration of experiment, days	Weight at start	Weight at end
6214	o		o		36 k	50	310
6215	o		o		10 d	40	46
6216	o		o		37 k	30	136
6217	+	37	o		37 k	44	230
6218	o		o		38 k	40	116
6219	o		o		37 k	44	158
6220	+	32	o		37 k	40	142
6221	o		o		37 k	40	160
6222	o		o		37 k	38	160
6223	o		o		37 k	40	110
6224	o		o		38 k	30	84
6225	o		o		36 k	44	180
6226	o		o		38 k	40	140
6227	o		o		37 k	40	150
6228	o		o		37 k	36	140

GROUP 139

Basal diet 97 without addition. Started Feb. 7, 1948

Chick no.	Exudate	Days	Encephalomalacia	Days	Duration of experiment, days	Weight at start	Weight at end
6281	+	26	o		26 d	34	66
6282	+	21	+	21	26 d	34	97
6283	+	21	o		37 k	36	240
6284	+	23	o		30 d	40	160
6285	o		o		34 d	34	70
6286	+	37	o		37 k	38	104
6287	o		o		37 k	40	100
6288	+	34	o		34 d	36	100
6289	+	24	o		30 d	32	70
6290	+		o		20 d	35	90

References p. 513.

GROUP 140

Basal diet 97 + 0.5 % ascorbic acid. Started Feb. 7, 1948

Chick no.	Exudate	Days	Encephalomalacia	Days	Duration of experiment, days	Weight at	
						start	end
6291	o		o		37 k	38	190
6292	o		o		37 k	34	194
6293	o		o		37 k	34	164
6294	o		o		37 k	38	190
6295	o		o		37 k	30	140
6296	o		o		36 d	36	160
6297	o		o		37 k	40	190
6298	o		o		37 k	30	174
6299	o		o		37 k	30	110
6300	o		o		37 k	32	180

GROUP 141

Basal diet 97 + 0.1 % ascorbic acid. Started Feb. 7, 1948

Chick no.	Exudate	Days	Encephalomalacia	Days	Duration of experiment, days	Weight at	
						start	end
6301	o		o		37 k	41	192
6302	o		o		37 k	44	183
6303	o		o		37 k	32	150
6304	o		o		37 k	44	140
6305	o		o		37 k	38	184
6306	o		o		37 k	40	140
6307	o		o		37 k	30	190
6308	o		o		19 d	42	54
6309	o		o		37 k	30	174
6310	o		o		37 k	46	156

GROUP 142

Basal diet 97 + 0.5 % nordihydroguaiaretic acid. Started Feb. 7, 1948

Chick no.	Exudate	Days	Encephalomalacia	Days	Duration of experiment, days	Weight at	
						start	end
6311	o		o		37 k	39	240
6312	o		o		35 d	38	102
6313	o		o		38 k	34	150
6314	o		o		38 k	40	210
6315	+	20	o		38 k	44	190
6316	o		o		38 k	46	170
6317	o		o		38 k	40	260
6318	o		o		37 k	44	210
6319	o		o		37 k	42	214
6320	o		o		38 k	45	200

References p. 513.

GROUP 143

Basal diet 124 without addition. Started Feb. 7, 1948

Chick no.	Exudate	Days	Encephalomalacia	Days	Duration of experiment, days	Weight at	
						start	end
6321	o		o		23 d	40	70
6322	o		+	16	21 d	30	80
6323	o		o		38 k	48	172
6324	o		o		18 d	40	80
6325	o		+	26	26 k	40	128
6326	o		+	17	26 d	30	60
6327	o		+	32	32 k	40	108
6328	o		o		19 d	39	52
6329	o		o		23 d	34	60
6330	o		o		12 d	40	27

GROUP 144

Basal diet 124 + 0.5% ascorbic acid. Started Feb. 7, 1948

Chick no.	Exudate	Days	Encephalomalacia	Days	Duration of experiment, days	Weight at	
						start	end
6331	o		o		38 k	40	179
6332	o		o		38 k	48	210
6333	o		o		10 d	44	55
6334	o		o		38 k	42	240
6335	o		o		38 k	34	198
6336	o		o		4 d	38	31
6337	o		o		38 k	48	172
6338	o		+	20	38 k	40	180
6339	o		o bl		38 k	40	200
6340	o		+	23	32 k	38	160

GROUP 145

Basal diet 124 + 0.5% cystine. Started Feb. 7, 1948

Chick no.	Exudate	Days	Encephalomalacia	Days	Duration of experiment, days	Weight at	
						start	end
6341	o		+	26	26 k	44	130
6342	o		o		12 d	40	53
6343	o		+	23	33 k	40	83
6344	o		+	27	28 k	44	90
6345	o		+	13	21 k	44	120
6346	o		o		38 k	48	172
6347	o		+	16	20 k	44	64
6348	o		o		21 d	38	114
6349	o		+	16	17 d	42	80
6350	o		+	32	38 k	45	190

The series of experiments started on Nov. 11, 1947 (groups 97, 98, and 100) were carried out with basal diet 97 + varying amounts of cystine. They showed that an addition of 0.5% cystine caused a decrease in the incidence of exudative diathesis, whereas as little as 0.05% had no such effect. This discovery was further examined in experiments started on Dec. 17, 1947 (groups 106, 107, 108, 109, and 110). Here again

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the groups containing additions of 0.5 and 1.0% cystine showed considerably less tendency towards exudation than the groups with 0.1% of cystine or less. In both series of experiments there seems to be a slightly increased tendency towards exudation in the groups with 0.05% of cystine as compared with those without added cystine.

The experiments started on Nov. 25, 1947 (groups 101 and 102) showed that the addition of 0.5% ascorbic acid definitely decreased the tendency to exudation. Not only were the number of cases with exudates low, but the exudates were of a much less marked type and occurred later than in the control group. Repetition of this experiment on Dec. 17 and Feb. 17 (groups 106 and 112, and 139, 140 and 141 respectively) showed complete depression of symptoms within the period of the experiments as a result of the addition of 0.5% and even 0.1% ascorbic acid*.

Experiments started Feb. 3, 1947 (groups 132 and 134) in which 0.05% of cystine was included in the diet confirmed the effect of 0.5% ascorbic acid in delaying and minimizing the tendency to exudation.

An experiment with nordihydroguaiaretic acid (NDGA) started Feb. 7, 1947 (group 142), to be compared with group 139) showed that this substance, in an amount of 0.5% of the diet, also definitely counteracted the symptom.

In the experiments reported so far no conclusions have been drawn as to the effect of the additions on encephalomalacia because of the relatively low incidence of this symptom.

Experiments with diets producing encephalomalacia were made partly with a diet containing a large amount of dried hog liver and partly with diets resembling that of PAPPENHEIMER AND GOETTSCH[5]. These experiments, started on Jan. 14, 1948, showed that 0.5% ascorbic acid did not depress the tendency to encephalomalacia with diets rich in hog liver (groups 122 and 123), whereas it did so in the case of diets resembling that of PAPPENHEIMER AND GOETTSCH (groups 124 and 125). A repetition of the latter experiments, Feb. 7, 1948, (groups 143 and 144) confirmed this finding, although a number of the animals in group 143 died before they had shown the symptoms. An experiment with 0.5% cystine added to diet 124 (group 145, to be compared with group 143) showed no effect on encephalomalacia.

In order to find out whether ascorbic acid and NDGA increase the amount of tocopherol in the tissue which is particularly affected by the exudates, we undertook a series of determinations of tocopherol in fat from adipose tissue. In order to get enough fat for the determinations in all cases samples of adipose tissue (subcutaneous + intraperitoneal) from several animals within the same group were pooled, and stored in the frozen state until the determination was made.

The method used is based on the procedure of EMMERIE AND ENGEL[6,7]. The details have been published stepwise in several papers[8,9,10,11].

The determination is carried out in 3 steps:

1. *Extraction of the fat and saponification*

The tissue is ground with 1½ times its weight of sodium sulphate and extracted on a glass filter with chloroform. The chloroform solution is evaporated *in vacuo*, dissolved in peroxide free ether, transferred to a separatory funnel, and saponified at room temperature with 4 n methyl alcoholic potassium hydroxide in an atmosphere of nitrogen (purified with pyrogallol) for 2 hours. The mixture

* The ascorbic acid in the blood of 5 animals of group 112 after 43 to 44 days on this diet was 35, 27, 38, 41, and 38 g per ml as determined by the method of ROE AND KUETHER[3] with the modification that glacial acetic acid was used in stead of 85% H_2SO_4, as proposed by BOLOMEY AND KEMMERER[4]. The blood (2 ml) was obtained by a neeole from the carotid artery.

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is diluted with water, the aqueous phase is removed and washed with ether, the combined ether phases are washed with water until they are neutral. then with dilute potassiumhydroxide (2 %) and again with water until neutral and dried over sodium sulphate. After filtration the residue is dissolved in 10 ml benzene.

2. *Removal of vitamin A from the unsaponifiable matter by chromatography*

The adsorption column is prepared as follows: about 2 g of Special Filtrol and ¼ g of stannous chloride are suspended in 5 ml conc. HCl, heated to the boiling point, and poured into an adsorption tube. The liquid is sucked down under slight vacuum, washed once with 5 ml absolute ethanol and 5 times with 5 ml benzene. After the third addition of benzene the filtration is interrupted and the Special Filtrol stirred up. After the fourth addition of benzene, the column is compressed carefully by a glass rod. Then benzene is again poured on and a disk of filterpaper is placed on the top.

An aliquot part of the benzene solution is drawn through the tube and the column washed with 4 times 5 ml benzene. The filtrate is evaporated in vacuo, and dissolved in ether.

3. *Distillation and colour reaction*

The ether solution is transferred to the bottom of a small cylindrical vacuum still[8]. The ether is carefully evaporated in vacuo, whereupon the still is connected to the high vacuum pump aggregate and the substance distilled at a pressure below 10^{-3} mmHg at 100° C for one hour. The distillate is transferred from the condensor into a 25 ml volumetric flask with benzene and ethanol. To the solution are added 1 ml 0.2 % $FeCl_3$ and 1 ml 0.5 % α-α'-dipyridyl in ethanol and the flask is filled up to the mark with ethanol. The intensity of the color is measured photometrically after 10 minutes.

If a Pulfrich photometer with filter S. 50 is used $E^{1\%}_{1cm}$ will be 360 for α-tocopherol.

Tocopherol added to fat before the saponification can be found to the extent of about 70 %.

The results are presented in Table II.

TABLE II

DETERMINATION OF VITAMIN E IN FAT OBTAINED FROM ADIPOSE TISSUE

Group	Diet	Number of chicks	Number of chicks with exudate	Days on diet	μg tocopherol per g fat	Peroxide value
132	97+0.05% cystine.	6	5	37	8	39.6
134	97+0.05% cystine+0.5% ascorbic acid .	13	3	37	60	0
135	97+0.05% cystine+0.01% ephynalacetate*	8	0	37	37	0
139	97.	3	2	37	15	17.5
140	97+0.5% ascorbic acid	9	0	37	110	1.82
141	97+0.1% ascorbic acid	9	0	37	34	0
142	97+0.5% nordihydroguaiaretic acid . . .	8	0	38	100	0
178	commercial chicken ration	4	0	37	118	
180	commercial chicken ration	4	0	36	135	0

* dl-α-tocopherol acetate, from F. HOFFMANN-LA ROCHE, A.G., Basel, Schweiz.

The figures seem to indicate that ascorbic acid and NDGA counteract the exudative diathesis by protecting the tocopherol content of the tissue.

In Table II the last column contains the peroxide values of the fat as determined by the method of KING, ROSCHEN AND IRWIN[12] adapted for use with relatively small quantities of animal tissue (DAM AND GRANADOS[13].)It is apparent that in the groups where no symptoms occurred the peroxide value of the pooled fat is zero, whereas in the groups where exudation followed by the development of discoloured fat has occurred a certain amount of peroxide formation has taken place. In group 140, where the average tocopherol content is high, there is, nevertheless, a demonstrable though very low peroxide value. This is due to the fact that the pooled fat from this group contains fat of one animal which has had some discoloration of the adipose tissue. It would

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have been interesting to have had individual tocopherol and peroxide values from each animal in this group.

DISCUSSION

Our results and conclusions are in agreement with the *in vitro* experiments of GOLUMBIC AND MATTILL[14], who found that ascorbic acid added to lard with or without added tocopherol (β-) resulted in improved keeping qualities as measured by the length of the induction period, the improvement being largest when both substances were present simultaneously. These authors also showed that addition of ascorbic to ethyl esters of lard fatty acids with added α-tocopherol further retarded the oxidation of the latter substance.

They also agree to some extent with the *in vivo* experiment of A. OVERMAN[15] who found that ascorbic acid feeding increased the resistance of fat (in rats) to rancidity.

Further, our results should be compared with the experiments of HANSON *et al.*[16]. These authors reared rats on a diet containing just enough vitamin E to secure reproduction. The offspring of these rats were given a vitamin E deficient diet containing lard rancidified to a peroxide value of 20 or more. At 100–150 days of age they were given single doses of α-tocopherol, ascorbic acid, γ-tocopherol or NDGA. Six days later the animals were sacrificed and the fat from the peritoneal adipose tissue was melted down and its induction period determined by O_2 absorption at 100° C and peroxide accumulation at 63° C.

They found that only the ingested α- and γ-tocopherol lengthened the induction period whereas the ingested ascorbic acid and NDGA were inactive in this respect.

The reason for the apparent discrepancy between their and our experiments may be due to the fact they gave only one dose of the substances, whereas we gave the substances every day and in larger amounts. Another possibility is that their animals may have been absolutely deprived of vitamin E so that a sparing action of ascorbic acid or NDGA could not make itself manifest.

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SUMMARY

Exudative diathesis in chicks produced by rearing them on diets rich in highly unsaturated fatty acids and low in vitamin E can be counteracted to a considerable extent by the addition of 0.5 % ascorbic acid, nordihydroguaiaretic acid or cystine to the diet.

Encephalomalacia in chicks produced by diets low in vitamin E of the type used by PAPPENHEIMER AND GOETTSCH can be counteracted by the addition of 0.5 % ascorbic acid but not by a corresponding amount of cystine.

Encephalomalacia produced by diets containing 35 % dried hog liver did not respond to treatment with ascorbic acid at the level of 0.5 % of the diet.

Determinations of tocopherol in fat of adipose tissue show that ascorbic acid and nordihydroguaiaretic acid protect this tissue against depletion of vitamin E.

RÉSUMÉ

La diathèse exudative produite chez les poulets en les alimentant avec un régime riche en acides gras fortement non saturés, et déficient en vitamine E, disparaît par addition au régime de 0.5 % d'acide ascorbique, d'acide nordihydroguaiarétique ou de cystine.

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L'encéphalomalacie produite chez les poulets soumis à un régime déficient en vitamine E, analogue au régime de PAPPENHEIMER ET GOETTSCH, disparaît par addition de 0.5 % d'acide ascorbique, mais non par la qualité équivalente de cystine.

L'encéphalomalacie produite par des régimes contenant 35 % de foie desséché de porc n'est pas sensible à l'addition de 0.5 % d'acide ascorbique au régime.

Les dosages du tocophérol dans la graisse du tissu adipeux montrent que l'acide ascorbique et l'acide nordihydroguaiarétique protègent ce tissu contre la carence en vitamine E.

ZUSAMMENFASSUNG

Durch Verfüttern einer Nahrung mit hohem Gehalt an stark ungesättigten Fettsäuren und niedrigem Vitamin E-Gehalt wurde an Hühnern eine exsudative Diathese hervorgerufen. Diese konnte durch einen Zuschuss von 0.5 % Ascorbinsäure, Nordihydroguajaretsäure oder Cystin gehemmt werden.

Encephalomalacie, die bei Hühnern durch Vitamin E-arme Diäten des von PAPPENHEIMER UND GOETTSCH benutzten Typs erzeugt wurde, konnte durch Zugabe von 0.5 % Ascorbinsäure, aber nicht durch die entsprechende Cystinmenge gehemmt werden.

Wurde die Encephalomalacie durch eine Kost hervorgerufen, die 35 % getrocknete Schweisleber enthielt, so reagierte sie nicht auf die Behandlung mit 0.5 % Ascorbinsäure.

Tokopherolbestimmungen in Fett aus Fettgewebe deuten darauf hin, dass Ascorbinsäure und Nordihydroguajaretsäure dieses Gewebe gegen Verarmung an Vitamin E schützen.

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