Vitamin E and stress

8.* Nutritional effects of dietary stress with silver in vitamin E-deficient chicks and rats

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1. When chicks were given a fat-free casein–gelatin diet and, after 2 weeks of age, 0·15 % silver acetate in the drinking water, they were found to have colourless exudates mainly in the pectoral region and partly in the peritoneal and pericardial spaces. Vitamin E and selenium, separately and together, failed to prevent this condition. Vitamin E was required together with methionine to prevent the condition. Methionine itself induced green staining of a few of the exudates.

2. When lard was added to the casein–gelatin diet and the chicks were also given Ag, some green exudates were found in addition to the colourless ones. Addition of vitamin E or Se or both prevented the green exudates, but raised the incidence of colourless exudates. Methionine enhanced the green exudate condition, but again when combined with vitamin E prevented both types of exudate.

3. A similar condition characterized by colourless exudates was induced by giving chicks diets based upon gelatin, yeast BPC, α-protein (with extra salts) or α-protein with gelatin. A torula yeast diet induced green exudates and haemorrhages.

4. All the basal diets, which were deficient in sulphur amino acids, produced dystrophy of the breast muscle. Four of these diets contained no fat. Some diets also induced dystrophy of the gizzard. Vitamin E and Se protected against both these lesions. Methionine was protective in all except the torula yeast diet.

5. Ethionine induced muscular dystrophy in chicks given a vitamin E-deficient diet adequate in sulphur amino acids. Additional methionine or vitamin E was protective, but cystine was not. Ethionine also produced a small incidence of green exudates and slight haemorrhages. This condition was prevented by vitamin E but not by methionine. The liver damage due to ethionine was not prevented by vitamin E, methionine or cystine.

6. Liver necrosis was induced in rats by giving them an 8·3 % casein diet and Ag, 130–1000 ppm, in the drinking water or the diet. Necrosis was produced even in the absence of dietary fat. Vitamin E and DPPD (*N,N'*-diphenyl-*p*-phenylenediamine) prevented necrosis, but adenine sulphate (0·25 %), methionine (0·15 %) and IONOX 330 (2,4,6-tri-(3′,5′-di-*tert.*-butyl-4′-hydroxybenzyl) mesitylene) did not. Se, 0·05 ppm, protected against 130 ppm Ag, but 1000 ppm Ag overcame the protective effect of 1 ppm Se. Similarly, 3 ppm cyanocobalamin was partly protective against 130 ppm Ag, but not against the higher concentration. Gold chloride (1000 ppm Au) had a mildly necrotic effect against which vitamin E did not protect. Neither copper sulphate (500 ppm Cu) not arsanilic acid (70 ppm As) induced liver necrosis.

7. A high intake of Se (20 ppm as sodium selenate) was necrogenic in rats given a 10 % casein diet; vitamin E and methionine did not protect. Vitamin E and cystine raised the low incidence of an exudative condition found in rats given 20 ppm Se. Methionine opposed this action of vitamin E.

8. It was concluded that exudative diathesis in chicks could be resolved into a simple exudative condition and a superimposed haemorrhagic condition. Ag is a pro-exudative factor. Vitamin E and Se are also pro-exudative for chicks given the casein–gelatin–lard–Ag treatment. Torula yeast, methionine, lard, Ag (with lard) and ethionine are all pro-haemorrhagic factors. Se and Ag have an antagonistic relationship in rats and chicks; in chicks, however, Se synergizes with Ag when the supply of methionine is limited.

We have carried out a number of investigations on the effect of dietary stress in vitamin E-deficient chicks and rats. In view of current concepts of lipid peroxidation

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and the well-known connexion between dietary fat and vitamin E, we have been particularly concerned with the production of disease signs in animals given fat-free diets. As Diplock, Green, Bunyan, McHale & Muthy (1967) found that silver was remarkably hepatotoxic in vitamin E-deficient rats and that its effects could be reversed by α-tocopherol and selenium, we have extended these studies on the rat and have also studied the effects in chicks, paying particular attention to the interactions between dietary factors such as Se, vitamin E and the sulphur amino acids.

EXPERIMENTAL

Dietary ingredients. Casein, 'low vitamin content', was purchased from Genatosan Ltd, soya-bean α-protein from the Chemurgy Division of Central Soya Inc., London, torula yeast from Lake States Yeast and Chemical Division of St Regis Paper Co., Rhinelander, Wisconsin, USA, and yeast BPC ('flake yeast') from United Yeast Co. Ltd, Croydon, Surrey.

The salt–vitamin mixture used in all the chick diets supplied salt mixture (Bunyan, Diplock, Edwin & Green, 1962) 6·75 %, vitamin mixture (Bunyan *et al.* 1962) 0·2 % choline dihydrogen tartrate 0·4 %, chlortetracycline 0·2 ppm and, in the form of a stabilized powder, vitamin A, 25 i.u./g and vitamin D_3, 3·3 i.u./g. Methyl oleate (OLME), cod-liver oil fatty-acid methyl esters (CLOME) and maize oil methyl esters (MOME) were prepared free of tocopherol, as described by Green, Diplock, Bunyan, McHale & Muthy (1967).

Chick diets. The basal vitamin E-deficient diets had the following percentage compositions. Diet 1: casein 15, gelatin 10, salt–vitamin mixture 7·35 and glucose 67·65. Diet 2: gelatin 25, DL-tryptophan 0·5, salt–vitamin mixture 7·35 and glucose 67·15. Diet 3: torula yeast 60, glycine 0·5, L-arginine 0·2, salt–vitamin mixture 7·35 and glucose 31·95. Diet 4: yeast BPC 40, gelatin 3, salt–vitamin mixture 7·35 and glucose 49·65. Diet 5: α-protein 12, gelatin 10, DL-tryptophan 0·13, DL-phenylalanine 0·2, salt–vitamin mixture 7·35 and glucose 70·32. Diet 6: α-protein 24, glycine 1, salt–vitamin mixture 7·35 and glucose 67·65. Diet 7: this consisted of diet 6 with 0·25 % DL-methionine added. Additions to all these diets were made by replacing glucose. With all seven diets, growth was limited by the sulphur amino acids (Table 1). In some experiments, further amounts of methionine were added to correct this deficiency, making some other amino acid limiting (for details see Table 1).

Rat diets. The vitamin E-deficient diet A 10 Y 3 (Bunyan, McHale & Green, 1963) contained 25 % dried brewer's yeast, 10 % casein and 3 % lard. The 8·3 % casein diet had the percentage composition: casein 8·3, vitamin mixture 0·4, salt mixture 5·33 and glucose 86. The vitamin and salt mixtures have been described by Diplock *et al.* (1967). Each rat was given 300 i.u. vitamin A palmitate per week by mouth. Additions were made by replacing glucose. Diet G 10 F had the percentage composition: casein 10, lard 5, salt mixture (Diplock *et al.* 1967) 5.33, vitamin mixture (Diplock *et al.* 1967) 0·4, sugar 59·3, and glucose 20. Vitamin A was added as a stabilized powder to give 11 i.u./g diet. Sodium selenite was added to give 0·03 ppm Se. Methionine and cystine were the limiting amino acids for growth on these diets

(Table 1). In some experiments (see Tables 7, 8), amounts of methionine were added to correct this deficiency by making some other amino acid limiting.

Chicks. Most tests were done with Rhode Island Red × Light Sussex cockerels and Warren cockerels purchased at 1 day of age from a commercial breeder. They were reared in electrically heated cages with wire floors and provided with food and water *ad lib.* In one experiment (Table 6) 1-day-old Cornish × Bilch pullets were used.

Rats. These were rats of the Norwegian hooded strain given, together with their dams, diet A10Y3 until the start of the experiments, which was usually at about 5 weeks of age.

Determination of total lipid and polyunsaturated fatty acids (PUFA)

The methods described by Bunyan, Murrell, Green & Diplock (1967) were used.

Table 1. *Amino acid contents of the vitamin E-deficient diets used*

	Basal diets			Basal diets supplemented with methionine	
No.	Protein and amino acid* constituents (%)	Met+cys content as % of requirement†	Second limiting amino acids*†	DL-met added (% of diet)	Total met (% of requirement†)
		Chick diets			
1	Casein 15, gelatin 10	64	trp (89)	0·5	116
2	Gelatin 25, DL-trp 0·5	20	leu (43)	0·5	70
	Gelatin 25, DL-trp 0·5+torula yeast 20	34	leu (59)	0·5	70
3	Torula yeast 60, gly 0·5, L-arg 0·2	69	arg/trp/leu (98)	0·6	113
4	Yeast BPC 40, gelatin 3	55	phe/tyr (74)	0·4	105
5	α-Protein 12, gelatin 10, DL-trp 0·13, DL-phe 0·2	24	leu (75)	0·6	94
6	α-Protein 24, gly 1	46	lys (95)	0·6	114
		Rat diets			
	Casein 8·3	52	thr (70)	0·15	76
	Casein 10 (diet G10F)	52	thr (70)	0·15	72

* Trp = tryptophan; gly = glycine; arg = arginine; phe = phenylalanine; leu = leucine; thre = threonine; tyr = tyrosine; lys = lysine; met = methionine and cys = cystine. Amino acid contents of proteins were obtained from Block & Weiss (1956).

† Percentage of requirement given for chicks by the National Research Council (1960) and for rats by Bender (1960).

RESULTS

Chick experiments

In all, about 1100 chicks were used in the studies of basal diets 1–6 and these diets with various additives (see Tables 2–4). The diets and treatments have been numbered in the tables and these numbers are included for clarity in the text below in parentheses. Most of the dietary treatments were examined more than once and the results have been combined for presentation. The tables show the number of chicks used for test of each of the main experimental treatments, but not the number used for the test of each additive, which was about fifteen chicks. The experiments with ethionine were done with groups of five or six chicks, as shown (Tables 5, 6).

Table 2. *Factors affecting the exudative condition in chicks given vitamin E-deficient diets containing casein and/or gelatin*

(Male chicks were given the diets from 1 day of age; survivors were killed at about 4 weeks of age)

Basal diet*		Other treatments or additions (%)	No. of chicks that died/ total	Colourless exudates			Green pectoral exudates and haemorrhagic condition	Effects† of various additives‡ on the exudative conditions							
No.	Protein constituents (%)			Pectoral	Peritoneal	Pericardial		Se	E	Se+E	Met	Met+Se	Met+E	Met+Se+E	Ethoxyquin
				(no. of chicks with exudates/total)											
1	Casein 15+gelatin 10	(*a*) None	0/18	0/18	—	—	—	—	—	—	—	—	—	—	—
							0/18	—	—	—	—	—	—	—	—
		(*b*) Lard 4	0/16	0/16	—	—	—	0	0	—	0	—	—	—	—
							0/16	0	0	—	0	—	—	—	—
		(*c*) Ag§	1/33	27/33‖	3/33	—	—	0	0	0	−1	−2	−2	−2	—
							0/33	0	0	0	+1	+1	0	0	—
		(*d*) Lard 4 and Ag§	2/44	15/44‖	5/44	—	—	+2	+2	+2	0	−2	−2	−2	+1
							8/44	−1	−2	−2	+1	+1	−2	−2	−2
2	Gelatin 25	(*a*) None	2/27	12/27	12/27	2/27	—	—	—	—	−1	—	—	—	—
							0/27	—	—	—	0	—	—	—	—
		(*b*) Lard 4	1/19	7/19	8/19	—	—	—	—	—	−1	—	—	—	—
							0/19	—	—	—	0	—	—	—	—
		(*c*) Torula yeast 20	1/18	5/18	—	—	—	—	—	—	−2	−2¶	−2	—	—
							0/18	—	—	—	+2	0¶	0	—	—
		(*d*) Lard 4+torula yeast 20	0/9	7/9	3/9	—	—	—	—	—	−1	—	—	—	—
							1/9	—	—	—	+2	—	—	—	—
		(*e*) Stripped maize oil 1	3/18	11/18	6/18	2/18	—	0	−2	—	−2	—	—	—	—
							0/18	0	0	—	0	—	—	—	—
		(*f*) Stripped maize oil 1+lard 4	0/9	6/9	6/9	—	—	−1	—	—	—	—	—	—	—
							0/9	0	—	—	—	—	—	—	—

* See p. 166 for details of these vitamin E-deficient diets.

† Type and degree of effect: +2, clearly aggravated the condition; +1, slightly increased the incidence of the condition; 0, no effect; −1, decreased the incidence; −2, eliminated the condition; —, not tested.

‡ Se = sodium selenite supplying Se (1 ppm unless stated); E = D-α-tocopheryl acetate (120 ppm); met = DL-methionine (0·5 % unless stated); ethoxyquin = 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline, 0·1 % of diet.

§ Silver acetate, 0·15 % of drinking water, from 2 weeks of age.

‖ Copious exudates.

¶ Se, 0·15 ppm, for this group.

The exudative condition in chicks. The factors affecting the production and prevention of the exudative condition in vitamin E-deficient chicks are set out in Tables 2 and 3. Diets containing casein and gelatin (1 *a*) or casein, gelatin and lard (1 *b*) did not induce the formation of exudates (Table 2). However, when chicks receiving the casein–gelatin diet were also given silver acetate in the drinking water (1 *c*), most of them were found to have copious colourless exudates in the pectoral region. Dietary Se (1 ppm) and/or vitamin E (120 ppm) had no protective effect. Methionine decreased the incidence of colourless exudates, but green exudates and haemorrhages appeared in a few chicks. Combination of methionine with Se almost completely eliminated the colourless exudates, but again allowed a few chicks to develop green exudates, whereas methionine + vitamin E, and methionine + vitamin E + Se completely eliminated exudates. Compared with the chicks given Ag and the lard-free diet (1 *c*), fewer of those given Ag and the lard-containing diet (1 *d*) developed exudates, but a proportion of the exudates were green and the chicks also had mild, widespread subcutaneous haemorrhages. Vitamin E and/or Se now greatly increased the colourless exudates. Vitamin E eliminated the green exudates, but Se was only partially effective. With lard in the diet (1 *d*), methionine failed to affect the colourless exudates, but slightly increased the green exudates. Colourless exudates were, however, eliminated by methionine combined with Se and/or vitamin E, but the combined effects of methionine and vitamin E were required for the prevention of the green exudates. Giving chicks the synthetic antioxidant ethoxyquin (6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline) resulted in no green exudates but rather more of the colourless type.

The Ag treatment depressed the growth of the chicks (mean weight 150 g at 4 weeks old, compared to 250–300 g) and usually imparted a green tinge to the kidneys. A few of the chicks with colourless exudates on the breast muscle also had exudates in the abdominal and pericardial spaces. The green exudates were never as intense in colour as those seen in chicks given the torula yeast diet (see p. 171), but were often associated with enlargement of the gall bladder.

Diets containing 25 % gelatin (2 *a*) or gelatin and lard (2 *b*) induced only a moderate formation of colourless exudates that was decreased by the addition of methionine. When the diet contained gelatin and torula yeast (2 *c*), the further addition of methionine converted a moderate incidence of colourless exudates into an even greater number of green exudates. Addition of methionine, together with either Se or vitamin E, obviated all the exudates. When lard and torula yeast (2 *d*) were given, methionine increased the green exudates and decreased the colourless ones. Lard and/or stripped maize oil (2 *e*, *f*) produced a fairly high incidence of colourless, but not green, exudates. Se had little effect on these exudates, but vitamin E and methionine protected in full. Chicks given the gelatin diet (diet 2) grew very poorly, reaching only about 50 g at 3 weeks of age. With torula yeast in addition, chicks grew to about 105 g and the further addition of methionine raised this to 180 g.

In the foregoing results, four examples can be found of the conversion of a colourless exudate condition into one of green exudates by the addition of methionine to the diet and two similar examples for methionine + Se. In contrast to this, Se and/or vitamin E had the reverse effect of converting the green exudates induced by the Ag

Table 3. *Factors affecting the exudative condition in chicks given vitamin E-deficient diets containing α-protein, torula yeast or yeast BPC*

(Male chicks were given the diets from 1 day of age; survivors were killed at about 4 weeks of age)

Basal diet*		Other treatments or additions (%)	No. of chicks that died/ total	Colourless exudates			Green pectoral exudates and haemorrhagic condition	Effects† of various additives‡ on the exudative conditions							
								Se					E (50) +	Met	
No.	Protein constituents (%)			Pectoral	Peritoneal	Pericardial		0·05	0·2	0·5	1·0	E (120)	Se (0·1)	0·4	0·6
				(no. of chicks with exudates/total)											
3	Torula yeast 60	(*a*) None	3/17	0/17	—	—	—	—	—	—	—	—	—	—	0
							9/17	—	—	—	—	—	—	—	0
		(*b*) Lard 4	4/18	1/18	—	—	—	—	—	—	—	—	—	—	0
							13/18	—	—	—	—	—	—	—	+1
		(*c*) Lard 4 + met 0·6	1/8	0/8	—	—	—	0	0	—	0	0	0	—	—
							7/8	−2	−2	—	−2	−2	−2	—	—
		(*d*) Lard 4 + met 0·6 and Ag§	1/7	0/7	—	—	—	0	0	—	0	—	—	—	—
							4/7	−1	−2	—	−2	—	—	—	—
4	Yeast BPC‖ 40	(*a*) None	14/26	9/26	2/26	3/26	—	—	0	0	—	−1	−2	−1	—
							5/26	—	−1	−2	—	−2	−2	−2	—
		(*b*) Lard 4	1/8	0/8	0/8	0/8	—	—	—	—	—	—	—	0	—
							2/8	—	—	—	—	—	—	0	—
5	α-Protein 12, gelatin 10	(*a*) None	3/5	4/5¶	4/5¶	—	—	—	—	—	—	—	—	—	−2
							0/5	—	—	—	—	—	—	—	0
6	α-Protein 24	(*a*) None	1/30	1/30	—	—	—	—	—	—	—	—	—	0	0
							0/30	—	—	—	—	—	—	0	0
		(*b*) Salt mixture 14	2/35	4/35¶	6/35¶	1/35¶	—	—	—	—	+1	0	—	—	−1
							0/35		—	—	0	0	—	—	0

* See p. 166 for details of these vitamin E-deficient diets.

† Type and degree of effect: +1, slightly increased the incidence of the condition; 0, no effect; −1, decreased the incidence; −2, eliminated the condition; —, not tested.

‡ Se = sodium selenite supplying Se (figures indicate ppm); E = D-α-tocopheryl acetate (figures indicate ppm); met = DL-methionine (figures indicate %).

§ Silver acetate in the drinking water from 2 weeks of age.

‖ Another batch of this yeast induced only a very small incidence of colourless exudates; mortality was also low.

¶ Copious exudates.

treatment into colourless exudates. The effect of Se depended upon its dietary concentration: 0·06 ppm was not active, but 0·2 and 1·0 ppm were. The entire elimination of the exudate conditions induced by Ag seemed to require both methionine and vitamin E. It did not seem to require Se.

Table 3 shows the results of experiments with diets containing torula yeast, yeast BPC or α-protein. With the torula yeast diet (diet 3), both lard (3*b*) and lard + methionine (3*c*) increased the incidence and severity of the green exudate–haemorrhage condition. The condition induced by these diets was much more severe than with any other diet used and the exudates were confined to the pectoral region; colourless exudates were never seen. As is well known, vitamin E and Se completely protect against the disease. It was also found that the Ag treatment (3*d*) opposed the protective effects of the lowest concentration of Se used, 0·05 ppm, allowing the formation of green exudates in three chicks and colourless exudates in one chick out of a group of nine. Another chick from this group had slight muscular dystrophy. Se at 0·2 ppm and 1·0 ppm overcame these effects and also prevented the growth depression produced by Ag. Chicks given Ag were only about 70 g in weight at 3 weeks of age compared to 150 g or more for controls.

Results for diet 4, containing yeast BPC, were variable. Green exudates were always found in a few chicks, but the colourless exudates were encountered in one experiment only, owing possibly to differences between two batches of yeast (4*a*). In this experiment, the addition of lard (4*b*) removed the colourless exudates at the expense of increasing the green exudate condition. Se had no effect upon colourless exudates but, at higher concentrations, it eliminated the green exudates. Vitamin E and methionine decreased or eliminated the exudative state.

Copious colourless exudates, preventable by methionine, were also produced by diet 5, containing α-protein and gelatin. This diet is very low in the sulphur amino acids, as shown by the group mean weights at 3 weeks of age, without and with added methionine, namely 56 and 177 g.

Diet 6, containing α-protein but no fat (6*a*), induced no exudates, but, when the salt mixture (see p. 166) was, on one occasion, accidentally included in the diet at 21 % (6*b*) instead of the usual 7 %, copious exudates were found that were made a little worse by Se (1 ppm) and partly alleviated by methionine. Very variable volumes of fluid were found; some chicks had 18–30 ml in the abdomen, 4–10 ml in the pericardial sac and 5–12 ml over the breast muscle. Dietary methionine raised the mean weight at 4 weeks from about 104 g on the basal diet to about 290 g. Additional salts decreased this latter value to about 220 g.

In addition to the two types of exudates described above and the signs of muscular dystrophy described below, the experimental diets induced a number of other, apparently minor, abnormalities, such as blotchy or pale livers and kidneys. These signs were not removed by the dietary additives that prevented the main disorders studied. During the experimental period, mortality was low among chicks given all the experimental diets, except diet 4, as shown in Tables 2 and 3. However, many of the severely affected chicks were near to death when the experiments were terminated.

Muscular dystrophy in chicks. The incidence of dystrophy of the pectoral muscle and

Table 4. *Production and prevention of dystrophy of the breast muscle and gizzard in vitamin E-deficient chicks*

(Male chicks were given the diets from 1 day of age; survivors were killed at about 4 weeks of age)

Basal diet*			Incidence of breast muscle dystrophy					Incidence of gizzard dystrophy		
No.	Protein constituents (%)	Other additions or treatments (%)	No of chicks affected/ total	Severity†	Eliminated by‡	Decreased by‡	Unaffected by‡	No of chicks affected/ total	Eliminated by‡	Decreased by‡
1	Casein 15, gelatin 10	(*a*) None	5/18	8·0	—	—	—	0/18	—	—
		(*b*) Lard 4	13/16	17·5	met, Se, E	—	—	0/16	—	—
		(*c*) Ag§	14/33	5·8	met, E, met+E, met+Se, E+Se	Se	—	10/33	Se, E, Se+E, met+Se, met+E	met
		(*d*) Lard 4 and Ag§	16/44	6·1	met+Se+E	Se, ethoxyquin Se (0·06)	—	16/44	met+Se+E	ethoxyquin met
2	Gelatin 25	(*a*) None	23/37	18	met	—	—	10/27	met	—
		(*b*) Lard 4	16/19	22	met	—	—	8/19	met	—
		(*c*) Torula yeast 20	17/18	17	met+E, met+Se (0·15)	met	—	8/18	met+Se (0·15), met+E	met
		(*d*) Lard 4+torula yeast 20	8/9	—	met	—	—	5/9	met	—
		(*e*) Stripped maize oil	9/18	—	met (0·4), Se, E	—	Se (0·15)	7/18	met (0·4), Se, E	Se (0·15)
		(*f*) Stripped maize oil+lard 4	7/9	—	Se	Se (0·15)	—	7/9	Se	Se (0·15)
3	Torula yeast 60	(*a*) None	8/17‖	—	—	met (0·6)	—	0/10	—	—
		(*b*) Lard	9/18‖	—	—	met (0·6)	—	0/9	—	—
5	α-Protein 12, gelatin 10	(*a*) None	2/5	6·0	met (0·6)	—	—	3/5	met (0·6)	—
6	α-Protein 24	(*a*) None	24/30	17	met	—	met (0·25)	7/30	met (0·25)	—
		(*b*) Salt mixture 14	24/35	12	met, Se, E	—	—	7/35	met, Se, E	—

* See p. 166 for details of these vitamin E-deficient diets.

† Sum of (incidence (%) of severe dystrophy × 0·3), (incidence of moderate dystrophy × 0·2) and (incidence of slight dystrophy × 0·1). Maximum value = 30.

‡ E = D-α-tocopheryl acetate, 120 ppm; Se = sodium selenite supplying Se (1 ppm unless stated): met = DL-methionine (0·5 % unless stated); ethoxyquin = 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline, 0·1 % of diet.

§ Silver acetate, 0·15 % of drinking water, from 2 weeks of age.

‖ Muscle haemorrhages in addition to the striations.

the gizzard in chicks given five of the basal vitamin E-deficient diets is shown in Table 4. Dystrophy of the breast muscle developed in some chicks given the fat-free casein–gelatin diet (diet 1*a*) and the incidence and severity were greatly increased by the addition of lard (1*b*). Supplements of methionine, Se and vitamin E gave full protection. Treatment with Ag depressed growth and raised the incidence of breast-muscle dystrophy in chicks given both diets (1*c*, *d*), although the lesions were generally less severe. A number of the chicks were also found to have dystrophy of the gizzard, characterized by large areas of white degenerate tissue, extending deep into the muscle. Dystrophy of the breast muscle in chicks given Ag was prevented by supplements of methionine, vitamin E, Se and combinations of these three substances, and by ethoxyquin. Se seemed to be less effective in the absence of lard. All these dietary supplements, except methionine, which was only partially effective, also protected against the gizzard lesion.

Dietary methionine also protected against dystrophy of the two tissues in chicks given diets containing gelatin (2*a*) or gelatin and lard (2*b*), but not fully when the diet contained gelatin and 20 % torula yeast (2*c*). Combinations of methionine with either Se (0·15 ppm) or vitamin E were, however, protective. Methionine alone seemed to be protective when the gelatin–torula yeast diet also contained lard (2*d*). The diets with gelatin and maize oil (2*e*, *f*) also induced lesions in breast muscle and gizzard, against which methionine, vitamin E and the higher level of Se were protective.

The torula yeast diet, both with and without lard (3*a*, *b*), seemed to induce a mainly haemorrhagic and only moderately dystrophic lesion of the breast muscle, as a late manifestation of the generalized exudative condition. A few chicks also developed slight striations of the leg muscles. Methionine did not completely prevent muscle degeneration even at 0·6 % of the diet. The diet with α-protein and gelatin (5*a*) also induced dystrophic lesions, preventable by methionine. A high incidence and severity of the two dystrophic lesions was also found in chicks given the fat-free α-protein diet (6*a*). Full protection was afforded by supplementation with methionine to the chicks' known requirement. Supplements of methionine, Se and vitamin E also prevented the lesions in chicks given the α-protein diet with additional salts (6*b*). The diets containing yeast BPC also induced breast-muscle dystrophy, in low incidence, preventable by Se or vitamin E (results not shown in the tables).

Toxicity of ethionine for chicks. In studying the toxicity of ethionine for chicks, we used a vitamin E-deficient diet that contained just sufficient methionine to prevent the occurrence of muscular dystrophy, so that any antagonism between the two amino acids could be demonstrated. Only one out of five male chicks given this diet had (slight) muscular dystrophy, compared with the high incidence of moderate lesions in those chicks given the diet containing insufficient methionine (Table 5).

Ethionine was given to the chicks at 0·05 % and 0·10 % of the diet from 14 to 32 days of age. No toxic signs were seen, except in two chicks with exudates in the group given cystine and ethionine, 0·05 %. The concentration of ethionine was then raised to 0·2 % in one group and to 0·4 % in four groups (see Table 5). At 0·2 %, ethionine induced severe muscular dystrophy in one chick and a slight lesion in another. At 0·4 % the incidence of dystrophy was a little greater and one chick also had exudative

Table 5. *Toxicity of ethionine for vitamin E-deficient cockerels*

(Male chicks were given the diets described from 1 day of age; survivors were killed and examined at 32 or 40 days)

Basal diet (0–40 days)	Additions* to basal diet (%)		No. of chicks	Age when killed (days)	Incidence of toxic signs			
	14–32 days	33–40 days			No. with muscular dystrophy†	No. with exudates and haemorrhages‡	No. with liver damage§	No. that died (33–40 days)
Dystrophy-producing diet‖	None	None	6	40	5	0	0	0
	None	None	5	40	1	0	0	0
	Eth (0·05)	Eth (0·2)	5	40	2	0	5	0
	Eth (0·05)+E (0·02)	—	5	32	0	0	0	0
Dystrophy-producing diet+met (0·25)¶	Eth (0·05)+met (0·2)	—	5	32	0	0	0	0
	Eth (0·05)+cys (0·2)	—	5	32	0	2	0	0
	Eth (0·1)	Eth (0·4)	6	40	3	1	6	2
	Eth (0·1)+E (0·02)	Eth (0·4)+E (0·02)	6	40	0	0	6	3
	Eth (0·1)+met (0·20)	Eth (0·4)+met (0·20)	6	40	0	2	6	2
	Eth (0·1)+cys (0·2)	Eth (0·4)+cys (0·2)	6	40	3	1	6	3

* Met = DL-methionine; eth = DL-ethionine; E = D-α-tocopheryl acetate; cys = L-cystine.

† Mainly striations of the breast muscle.

‡ Green exudates in the pectoral region; haemorrhages in the pectoral and peritoneal cavities.

§ Pale, 'fatty' livers, some with red specks.

‖ Diet 7 (α-protein 24 %, DL-methionine 0·25 %, see p. 166).

¶ The additional methionine made the diet adequate in sulphur amino acids.

Table 6. *Toxicity of ethionine for vitamin E-deficient pullets*

(Female chicks were given the diets described from 1 day of age. All chicks survived to 35 days of age, when they were killed and examined)

Basal diet (0–35 days)	Addition* to basal diet (%) (14–35 days)	No. of chicks	Mean weight gain, 14–35 days (g)	Incidence of toxic signs†			Liver lipid (%)	Liver PUFA‡ (mg/g)
				No. with muscular dystrophy	No. with exudates and haemorrhages	No. with liver damage		
Dystrophy-producing diet§	None	6	300	6	0	0		
Dystrophy-producing diet+met (0·25)‖	None	5	356	0	0	0	7·2	3·6
	Eth (0·025)	6	384	0	1	0		
	Eth (0·050)	6	352	0	1	6		
	Eth (0·10)	6	330	0	2	6		
	Eth (0·20)	6	187	2	2	6	10·8	7·3

* Met = DL-methionine; eth = DL-ethionine.

† Striations of the breast muscle; green exudates in the pectoral region; haemorrhages in the pectoral region and peritoneal cavity; fatty livers, some with red specks.

‡ Polyunsaturated fatty acids.

§ Diet 7 (α-protein 24%, DL-methionine, 0·25%, see p. 166).

‖ The additional methionine made the diet adequate in sulphur amino acids.

diathesis. The dystrophic lesions due to 0·4 % ethionine were prevented by vitamin E and methionine, but not by cystine. Vitamin E prevented the exudate formation, but methionine and cystine did not. Ethionine, at 0·2 or 0·4 %, induced weight losses and liver and kidney damage in all the chicks, even in those given vitamin E, methionine or cystine.

Female chicks did not seem much more sensitive to ethionine than the males (Table 6). Ethionine at 0·2 % produced slight muscular dystrophy and exudates in a few chicks and the lower concentration of ethionine induced exudates in one or two chicks per group.

Rat experiments

Effects of silver, gold, arsenic and copper in rats. Table 7 shows the toxic effects of Ag given to young rats receiving various vitamin E-deficient diets. The prolonged addition of 1000 ppm Ag to the drinking water of rats receiving diet A 10 Y 3 (containing 10 % casein and 25 % dried brewer's yeast) had no harmful effects. However, this concentration of Ag rapidly induced a high incidence of liver necrosis in rats given a diet with 8·3 % casein as the only protein; the rats usually died within about 14 days. It was also found that as little as 130 ppm Ag in the drinking water or the diet had the same effect. The necrogenic effect of Ag was evident regardless of the absence or presence of dietary lipid of whatever degree of unsaturation. A small incidence of liver necrosis was found in rats given the various basal diets (except A 10 Y 3), suggesting that the concentration of Se in the casein at 8·3 % of the diet was barely adequate nutritionally. Vitamin E, at 50–120 ppm, and 100 ppm DPPD(*N*,*N*′-diphenyl-*p*-phenylenediamine) prevented necrosis, but 0·15 % DL-methionine and 200 ppm IONOX 330 (2,4,6-tri-(3′,5′-di-*tert.*-butyl-4′-hydroxybenzyl) mesitylene) did not. Adenine sulphate (0·25 %), which prevents the fatty liver due to feeding rats on 1 % orotic acid (Creasey, Hankin & Handschumacher, 1961), was also ineffective against Ag poisoning. The degree of protection afforded by Se depended upon the concentration of Ag; 1 ppm Se did not completely protect rats given 1000 ppm Ag, whereas as little as 0·05 ppm Se protected against the effects of 130 ppm Ag. Similarly, cyanocobalamin partially opposed the effect of Ag at 130 ppm but not at 1000 ppm. Cu was tested only at 500 ppm, but it did not induce liver necrosis. Histological examination showed slight necrotic changes in the livers of rats given Au at 330 and 1000 ppm. At the higher concentration, the lesions were visible to the naked eye. However the lesions produced by Au did not prove rapidly fatal as found for Ag. In fact, only one rat died out of the group of seven, during the 8-week feeding period. Vitamin E did not protect against these liver lesions. Au did not affect the kidney.

Since As is known to antagonize the toxic effects of high levels of Se, it was thought possible that it might also oppose low concentrations and thus induce liver necrosis, by producing a deficiency of Se, in rats given the 8·3 % casein diet. However, As produced no signs of liver necrosis when given at 70 ppm, as arsanilic acid.

Toxic effects of Se in the rat. The toxic effects of Se, at 20 ppm, given as sodium selenate, are shown in Table 8. Most of the rats died, half of those that did having a necrotic lesion of the liver. One rat also had an exudative condition. Vitamin E, methionine or cystine, or combinations of these, failed to protect against the liver

Table 7. *Effects of silver, gold, arsenic and copper in rats*

(Weanling vitamin E-deficient rats were given the treatments described below from about 33 days of age; survivors were killed and examined at about 70 days of age)

Basal diet	Additive*	Concentration (ppm)	Route of administration	No. of rats with macroscopic signs of liver necrosis/ total	Substances† giving complete protection	Substances† giving partial protection	Substances† giving no protection
A 10 Y 3‡	None	—	—	0/6	—	—	—
	Ag	1000	Drinking water	0/6	—	—	—
Casein diet§	None	—	—	3/13	—	—	—
	Ag	130	Drinking water	4/7	—	—	—
	Ag	1000	Drinking water	5/6	E (120)	—	—
Casein diet§ + coconut oil 8	None	—	—	0/6	—	—	—
	Ag	1000	Drinking water	5/5	(E 120)	Se (1)	—
Casein diet§ + OLME 5	None	—	—	0/6	—	—	—
	Ag	1000	Drinking water	6/6	E (120)	Se (1)	met (0·15 %)
Casein diet§ + lard 10	None	—	—	—	—	—	—
	Ag	130	Drinking water	4/4	—	B_{12} (3)	Adenine sulphate (0·25 %)
	Ag	130	Diet	7/7	—	—	—
	Au	1000	Diet	2/7‖	—	—	E (200)
	As	70	Diet	0/8‖	—	—	E (200)
Casein diet§ + cod-liver oil 6 + lard 2	None	—	—	5/23	—	—	—
	Ag	1000	Drinking water	22/23	E (120) DPPD (100)	Se (1)	Se (0·5), met
Casein diet§ + CLOME 2·5 + MOME 2·5	None	—	—	1/29	—	—	—
	Ag	3–30	Drinking water	4/21	—	—	—
	Ag	130	Drinking water	10/13	Se (1·0 and 0·05)	—	—
	Ag	190–520	Drinking water	10/10	—	—	—
	Ag	1000	Drinking wtaer	41/42	E (50)	Se (1)	E (10), met (0·15 %), B_{12} (3), IONOX 330 (200)
	Au	330	Diet	0/6	—	—	—
	Cu	500	Diet	1/6	—	—	—

* Ag as silver acetate, Au as gold chloride, As as arsanilic acid and Cu as cupric sulphate.

† E = D-α-tocopheryl acetate; Se = selenium as sodium selenite; met = DL-methionine; B_{12} = cyanocobalamin; DPPD = *N*,*N*′-diphenyl-*p*-phenylenediamine; IONOX 330 = 2,4,6-tri-(3′,5′-di-*tert.*-butyl-4′hydroxybenzyl) mesitylene. Figures in parentheses indicate ppm, unless otherwise stated.

‡ See p. 166 for details. Cod-liver oil (10 %) was added for the last 6 weeks of this 16-week feeding experiment.

§ See p. 166 for details of this fat-free, vitamin E-free diet containing 8·3 % casein. OLME = methyl oleate, CLOME = cod-liver oil methyl esters and MOME = maize oil methyl esters (see p. 166).

‖ Four rats of each group were taken for histological examination at 60 days of age (see p. 176). The remaining three or four rats were kept on diet until 89 days of age.

lesion or alleviate the loss of weight caused by Se. Additions of vitamin E and/or cystine induced a much greater incidence of the exudative condition that was seen in only one of the rats given Se alone. Methionine protected against the exudative condition, even when given with vitamin E.

Table 8. *Toxicity of sodium selenate for rats*

(Weanling vitamin E-deficient rats were given the treatments described below from 35–43 days of age. Survivors were killed and examined at 80 days of age)

Addition to diet G10F*	No. of rats	No. of rats that died	No. of rats with liver necrosis	No. of rats with exudates†	No. of rats with interstitial oedema of mesenteric fat
None	11	0	0	0	0
E	5	0	0	0	0
Se	13	10	5	1	0
Se+E	11	6	7	6	4
Se+met	6	4	4	1	1
Se+cys	6	2	4	4	4
Se+met+E	6	3	4	0	1
Se+cys+E	6	3	4	3‡	3

* See p. 166 for details of this vitamin E-deficient diet. E = D-α-tocopheryl acetate, 500 ppm; Se = selenium, 20 ppm, as sodium selenate; met = DL-methionine, 0·6 %; cys = L-cystine, 0·6 %.

† Variously seen in the thorax, abdomen and subcutaneous spaces. Usually colourless, sometimes slightly green.

‡ Copious exudates (6, 16 and 24 ml) in these rats of about 75 g body-weight.

DISCUSSION

Exudative diathesis was discovered by Dam & Glavind (1938, 1939) and further investigated and described by Dam (1944). The disease was first produced by using diets containing casein and cod-liver oil and then later by diets containing torula yeast (Scott, Hill, Norris, Dobson & Nelson, 1955; Bieri, Pollard & Briggs, 1957). The exudates produced by such diets were reported to be 'often green' in colour owing to decomposition products of haemoglobin and they only rarely occurred in the pericardial or peritoneal cavities. Our experience with the torula yeast diet confirms this; in fact, in our experiments the exudates were almost always green in colour and confined to the regions over the breast and leg muscle or, occasionally, along the underpart of the wing. A similar description was given by Hartley & Grant (1961) for field outbreaks of exudative diathesis. Dietary supplements of either Se or vitamin E protected completely against the disease but, as we have shown (Table 3), it was not prevented by amounts of methionine sufficient to avoid nutritional deficiency of the sulphur amino acids. Cheville (1966) has investigated the pathology of exudative diathesis in chicks given a torula yeast diet (60 % yeast, 3 % stripped maize oil and 0·22 % methionine) and found the early gross lesions to be due to diffuse subcutaneous, intermuscular and interstitial oedema. Hydropericardium was common in his chicks, but not in ours. Cheville also found that a diet containing 52 % torula yeast, 5 % stripped maize oil and 1 % methionine induced both the exudative condition and dystrophy of the pectoral and gizzard muscles. Muscular dystrophy was the pre-

dominant syndrome in chicks given 54 % torula yeast, 0·5 % stripped maize oil and 1 % methionine.

Bird & Culton (1940) noted exudates in the peritoneal and pericardial spaces of chicks given diets containing skim milk and cod-liver oil. Later, Bird (1943) found that dietary NaCl, but not KCl, enhanced the oedema formation due to the ability of Na^+ to accumulate in the extracellular fluid. The partially curative effects of cystine against the exudative condition resulting from giving chicks a diet containing casein, dried brewer's yeast and 10 % cod-liver oil were reported by Dam, Kruse, Prange & Søndergaard (1948). Gitler (1958) studied these and other features of the exudative condition, and also concluded that high Na^+ content was the main dietary factor that promoted the appearance of exudates. Reiser (1950) discovered colourless exudates in some chicks given a fat-free diet containing 35 % α-protein and adequate methionine and vitamin E. However, the α-protein in use then may have contained some residual sodium bisulphite. Miller, Small & Norris (1955) found that α-protein from the same source contained 0·6–1·2 % sodium bisulphite and that this substance destroyed vitamin E and induced exudative diathesis. The α-protein used in our experiments contained only 0·14 % sodium bisulphite and did not induce exudates except when additional salt mixture was present in the diets.

Even in the light of these diverse findings, the disease pattern produced in our experiments by the Ag treatment of chicks given the casein–gelatin diet would seem to be novel in several respects. When the diet contained no fat, the exudates were entirely colourless and a small proportion of them were found to occur in the peritoneal cavity as well as over the breast muscle. Whilst vitamin E and Se failed to prevent these exudates, methionine decreased the incidence somewhat, but also caused some of them to be stained green. Protection against the exudative condition seemed to require the combined effects of methionine and vitamin E. Se reinforced the protective action of methionine against colourless exudates but failed to oppose its ability to promote green staining. In addition, the disease pattern was markedly changed by the addition of lard to the diet. The exudates in about one-third of the affected chicks were then green, although not so intensely coloured as in chicks given the torula yeast diet. Vitamin E or Se, or both, now had the striking effect of preventing the green exudates (the effect of Se alone was only partial) and also, at the same time, of increasing the incidence and severity of the colourless exudates. Addition of methionine seemed to enhance the green exudates, and also partly to overcome the protective effect of Se. In the presence of dietary fat, as found above for fat-free diets, methionine + Se, or methionine + vitamin E, were necessary for the removal of the colourless exudates, whereas only the latter combination protected against green exudates as well. It is remarkable that the exudate-promoting agents, Se and vitamin E, could combine with methionine, which had only a partially protective effect on its own, to provide complete protection. The action of ethoxyquin, with lard in the diet, was similar to that of vitamin E.

These studies confirm the proposal of Dam, Nielsen, Prange & Søndergaard (1958*a*) that Ag can act as a stress factor for the production of exudative diathesis. Ag seems to oppose the action of Se, as shown in the studies with the torula yeast diet.

Lard and methionine promote the appearance of green exudates in chicks given the torula yeast diet, as they also do in chicks given the casein–gelatin diet and Ag.

The experiments with yeast BPC confirmed the contention of Dam, Nielsen, Prange & Søndergaard (1958*b*) that green exudates could be produced by a diet that was low in polyenoic fatty acids. However, we also found, with one batch of the yeast, a moderate incidence of colourless exudates, not reported by Dam *et al.* (1958*b*). In keeping with the results above, Se protected against the green exudates, but not, at 0·2 ppm, against the other type.

The deficiency state produced by the gelatin diets showed several features in common with the results for the casein–gelatin–Ag treatments; colourless exudates were produced, some in the pericardial sac. Methionine was partly protective, but in the presence of torula yeast it promoted green exudates. Se failed to alleviate the condition produced by the diet with maize oil. Some of the most copious colourless exudates were produced by the fat-free, vitamin E-free diets containing 12 % α-protein and 10 % gelatin, or 24 % α-protein and extra salts. Methionine was fully or partially protective but Se and vitamin E failed to protect.

Most of the diets used in this study of exudates also induced muscular dystrophy. Four of the diets were fat-free. In two of these diets, fat proved to be an additional stress factor. These results agree with those of Hutcheson, Hill & Jenkins (1963). Dietary methionine, Se or vitamin E all protected against the lesions, although the effect of Se could be overcome by sufficient Ag. The lesions produced by the torula yeast diet seemed different in that they were largely haemorrhagic and also were not prevented by methionine. Dystrophy of the gizzard was found in chicks given the α-protein and gelatin diets; methionine, Se or vitamin E were protective. The torula yeast diet did not induce this lesion, nor did the casein–gelatin diet, unless the chicks were also given Ag. Se and vitamin E protected against this treatment, but methionine was only partially effective.

In addition to causing liver damage, ethionine also induced muscular dystrophy in some chicks, presumably by antagonizing methionine, for additional methionine was protective. Cystine did not prevent this action of ethionine, as it might have been expected to do in view of the contention of Scott & Calvert (1962) that cystine rather than methionine has a specific role in prevention of muscular dystrophy. Ethionine also induced some green exudates. This may have been due to antagonism of Se since methionine did not protect these chicks.

In the rat, Ag acts mainly as a liver poison, against which vitamin E, DPPD and dietary protein, at high concentration, are protective. Se competitively overcomes the necrogenic effect of Ag in the rat but methionine is not effective. Cyanocobalamin has been found to decrease the hepatotoxic action of carbon tetrachloride (Hove & Hardin, 1950) and we have found it to be partially protective against a lower level of Ag. Au, at the higher level, produced some signs of liver necrosis, but this lesion did not prove fatal, as was found for Ag poisoning at much lower concentrations. Cu, at 500 ppm, was not necrogenic. At a high level of intake, Se itself showed necrogenic properties in the rat and vitamin E and sulphur amino acids did not oppose this action. Se also showed some exudate-producing power, enhanced by vitamin E and

cystine and decreased by methionine. Morss & Olcott (1967) also found that vitamin E did not decrease the acute oral toxicity of sodium selenite. Sellers, You & Lucas (1950) described liver damage due to Se in rats given an adequate diet; ascites was found in association with the more severely damaged livers.

No clear overall picture emerges from the various studies on the interrelationship between Ag, Se, vitamin E and the sulphur amino acids in rats and chicks. However, our results do suggest that exudative diathesis in chicks can be resolved into two separate conditions. The first, which can be found on its own, is a simple exudative condition, probably resulting from increased permeability of the capillaries. The second is a haemorrhagic state that always seems to be superimposed on the exudative condition, the exudates then being green probably owing to decomposition products of porphyrins. Several pro-exudative factors can be listed. These include the α-protein diet with extra salts, the casein–gelatin diet with the added stress of Ag, and the basal diets containing gelatin or α-protein and gelatin. The inactivity of the casein–gelatin and α-protein basal diets themselves suggests that a delicate balance of pro- and anti-exudative factors is involved. Vitamin E and Se are also pro-exudative for chicks given the casein–gelatin–lard–Ag treatment. The list of pro-haemorrhagic factors must include the mineral and lipid components of torula yeast studied by Bieri, Briggs & Pollard (1958) as well as those found in our experiments: methionine, lard, Ag (in the presence of lard) and ethionine. In the rat, Se itself, at high levels, is the only pro-exudative factor discovered. In both rats and chicks, Ag antagonizes the effects of Se. However, in chicks, this antagonism is only shown in the presence of adequate methionine; when methionine is limiting for growth, Se synergizes with Ag in its pro-exudative action. This newly found involvement of methionine in exudative diathesis brings the disease more in line with muscular dystrophy in chicks, in which disease deficiencies of the same three nutrients, methionine, Se and vitamin E, are implicated. It is of interest that Century & Horwitt (1964) have discovered a role for Se in delaying the onset of encephalomalacia, a disease formerly ascribed to uncomplicated vitamin E deficiency.

It is well-known that dietary unsaturated lipid accelerates the onset of vitamin E-deficiency diseases and this fact has been important in the formulation of the antioxidant hypothesis of the action of vitamin E. However, many other substances can act similarly as stress factors, as we have shown, and the antioxidant hypothesis seems inadequate as an explanation of all these effects.

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REFERENCES

Bender, A. E. (1960). *Clinica chim. Acta* **5**, 1.
Bieri, J. G., Briggs, G. M. & Pollard, C. J. (1958). *Proc. Soc. exp. Biol. Med.* **99**, 262.
Bieri, J. G., Pollard, C. J. & Briggs, G. M. (1957). *Fedn Proc. Fedn Am. Socs exp. Biol.* **16**, 381.
Bird, H. R. (1943). *Science, N.Y.* **97**, 98.
Bird, H. R. & Culton, T. G. (1940). *Proc. Soc. exp. Biol. Med.* **44**, 543.
Block R. J. & Weiss, A. B. (1956). *Amino Acid Handbook*. Springfield, Illinois: Charles C. Thomas.
Bunyan, J., Diplock, A. T., Edwin, E. E. & Green, J. (1962). *Br. J. Nutr.* **16**, 519.
Bunyan, J., McHale, D. & Green, J. (1963). *Br. J. Nutr.* **17**, 391.

Bunyan, J., Murrell, E. A., Green, J. & Diplock, A. T. (1967). *Br. J. Nutr.* **21**, 475.
Century, B. & Horwitt, M. K. (1964). *Proc. Soc. exp. Biol. Med.* **117**, 320.
Cheville, N. F. (1966). *Path. Vet.* **3**, 208.
Creasey, W. A., Hankin, L. & Handschumacher, R. E. (1961). *J. biol. Chem.* **236**, 2064.
Dam, H. (1944). *J. Nutr.* **27**, 193.
Dam, H. & Glavind, J. (1938). *Nature, Lond.* **142**, 1077.
Dam, H. & Glavind, J. (1939). *Nature, Lond.* **143**, 810.
Dam, H., Kruse, I., Prange, I. & Søndergaard, E. (1948). *Biochim. biophys, Acta* **2**, 501.
Dam, H., Nielsen, G. K., Prange, I. & Søndergaard, E. (1958*a*). *Nature, Lond.* **182**, 802.
Dam, H., Nielsen, G. K., Prange, I. & Søndergaard, E. (1958*b*). *Experientia* **14**, 291.
Diplock, A. T., Green, J., Bunyan, J., McHale, D. & Muthy, I. R. (1967). *Br. J. Nutr.* **21**, 115.
Gitler, C. (1958). Studies on vitamin E deficiency with torula yeast diets in the rat and the chick. PhD thesis, University of Wisconsin, Madison, USA
Green, J., Diplock, A. T., Bunyan, J., McHale, D. & Muthy, I. R. (1967). *Br. J. Nutr.* **21**, 69.
Hartley, W. J. & Grant, A. B. (1961). *Fedn Proc. Fedn Am. Socs exp. Biol.* **20**, 679.
Hove, E. L. & Hardin, J. O. (1950). *Fedn Proc. Fedn Am. Socs exp. Biol.* **9**, 362.
Hutcheson, L. M., Hill, D. C. & Jenkins, K. J. (1963). *Poult. Sci.* **42**, 846.
Miller, R. F., Small, G. & Norris, L. C. (1955). *J. Nutr.* **55**, 81.
Morss, S. G. & Olcott, H. S. (1967). *Proc. Soc. exp. Biol. Med.* **124**, 483.
National Research Council (1960). *Publs natn. Res. Coun., Wash.* no. 827.
Reiser, R. (1950). *J. Nutr.* **42**, 319.
Scott, M. L. & Calvert, C. C. (1962). *J. Nutr.* **77**, 105.
Scott, M. L., Hill, F. W., Norris, L. C., Dobson, D. C. & Nelson, T. S. (1955). *J. Nutr.* **56**, 387.
Sellers, E. A., You, R. W. & Lucas, C. C. (1950). *Proc. Soc. exp. Biol. Med.* **75**, 118.

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