Metabolism of vitamin E in sheep*

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1. The metabolism of tritium-labelled tocopherol was investigated in thirty-six wethers and forty-two ewes fed on hay known to produce nutritional muscular dystrophy (NMD).

2. In one experiment, the effect of selenium or vitamin E or both on the metabolism of tritium-labelled tocopherol was studied. No difference was found in the incorporation rate of radioactivity in tissues due to the addition of either of these components.

3. In a second experiment, significantly more radioactivity was found in the tissues of sheep dosed intramuscularly than of those dosed by stomach tube into the rumen. Excretion of radioactivity was rapid when labelled tocopherol was given into the rumen.

4. In a third experiment, the efficiency of vitamin E in preventing NMD in lambs was tested. Supplementation with vitamin E of the diet consisting of NMD-producing hay and given to ewes was ineffective as a prophylactic treatment. Direct administration of vitamin E to the lambs completely prevented the occurrence of NMD. Plasma vitamin E concentrations in the treated lambs were significantly higher than in untreated lambs at weekly sampling over a 4-month period regardless of whether the lambs were born from ewes treated with the vitamin or not.

The metabolic interrelationships of selenium and vitamin E have received considerable attention during the last few years. Calvert, Nesheim & Scott (1962) have suggested a synergistic effect between vitamin E and selenium in the prevention of muscular degeneration in the chick. Desai & Scott (1965) reported that Se is effective in increasing the retention of radioactive tocopherol in the plasma of chicks. However, Cheeke & Oldfield (1969) did not confirm these results in rats and suggested further investigation to determine if there are species differences in the effect of Se on vitamin E metabolism. Thus, the work now reported was undertaken to study the relation of Se to vitamin E retention in sheep.

The absorption and retention of vitamin E administered to sheep either by stomach tube into the rumen or by injection also appeared to merit investigation. Investigations on monogastric animals by Overman, McNeely, Todd & Wright (1954), Gloor, Weber, Wunsch & Wiss (1963) and Imbesi (1958) indicated that oral administration of tocopherol was more effective than the parenteral route in increasing the concentration of tocopherol in the plasma. In contrast, Caravaggi, Gibbons & Wright (1968) reported that the plasma tocopherol concentrations in sheep were higher after intramuscular than after oral administration, and Brüggemann & Niesar (1954) reported that there was no increase of plasma tocopherol in sheep 14 d after intramuscular injection of vitamin E.

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EXPERIMENTAL

Analytical methods

The tissues were homogenized in 25 ml chloroform–methanol (2 : 1) per g of tissue and the lipids were extracted several times with a mixture of chloroform–methanol (2 : 1). After evaporation of the solvent, the lipids were dissolved in 10 ml of scintillation solution (60 mg 2,5-diphenyloxazole and 1 mg 1,4-di-2-(5-phenyloxazolyl) benzene in toluene) and counted in a Packard liquid scintillation counter 518. All values were converted into disintegrations/min (dpm) by internal standardization.

Expt 1. Twenty 4-month-old wethers were given hay from an area in northern Ontario where nutritional muscular dystrophy (NMD) is common. Because the hay was low in both energy and nitrogen, 2% urea and 6% sucrose were added to each day's feed supply by spraying in a water solution over the hay. All wethers received vitamin A (10 000 i.u.) and vitamin D (1750 i.u.) at weekly intervals. The wethers were divided for experimental purposes into four groups, each of five animals, and received the NMD-producing hay either unsupplemented, or were given weekly, by stomach tube into the rumen, a dose of 1 mg sodium selenite, or 1 g α-tocopherol, or 1 mg sodium selenite plus 1 g α-tocopherol. After 1 year on these treatments, all sheep, taken one per treatment at a time, were given by stomach tube into the rumen a dose of $4{\cdot}93 \times 10^7$ dpm/kg body-weight [^{3}H]DL-α-tocopherol-(5-methyl-T) in aqueous ethanol solution. The radioactive tocopherol (430 mCi/m-mole) was purchased from Radiochemical Centre, Amersham, England. Blood samples were taken from each sheep 2, 4, 6, 10 and 24 h after administration of the radioisotope. All tissue samples were frozen and stored at −20° until analysed.

Expt 2. Sixteen Shropshire wethers were fed for 9 months on a NMD-producing hay. The animals were then placed in metabolism cages a few days before dosing and allowed hay and water *ad lib*. The tritium-labelled α-tocopherol, in aqueous ethanol solution, was administered once, either via stomach tube into the rumen or intramuscularly into gluteal muscle, at doses that varied between $8{\cdot}88 \times 10^8$ dpm and $2{\cdot}22 \times 10^9$ dpm from trial to trial. At each time of dosing, a pair of sheep of equal weight was dosed; one sheep intraruminally and the other intramuscularly with the same amount of radioactivity.

From the four sheep killed at 24 h, blood was collected hourly up to 12 h and then at 24 h after dosing. Urine and faeces, if any, were collected at the same time as blood. In the other trials, blood, faeces and urine were collected at daily intervals. The collected material was handled for counting as in the previous experiment.

Expt 3. Forty-two ewes fed on a NMD-producing hay for 3 years were used for this experiment. At 2 months of gestation, twenty-three of them (group B) were given, by stomach tube into the rumen, at weekly intervals a dose of 1 g of DL-α-tocopherol; the remaining nineteen ewes (group A) were given no treatment. Four ewes in group B and five in group A produced twin lambs. From birth to weaning, eleven lambs from each of groups B and A were treated each week orally with 500 mg of DL-α-tocopherol, while the remaining sixteen of group B and thirteen from group A were untreated.

Serum vitamin E concentrations were determined weekly by the method of Desai (1968) and serum aspartate aminotransferase (L-aspartate:2-oxoglutarate aminotransferase; *EC* 2.6.1.1) activities were determined every 2 weeks by the method of Reitman & Frankel (1957).

The carcasses of all lambs, whether the animals died or were killed, were examined for gross and microscopic lesions of NMD.

RESULTS AND DISCUSSION

Expt 1. The results showed a wide variation in the distribution of radioactivity in the tissues, even between animals on the same treatment. Since the standard deviation was largely proportional to the mean, it was necessary to use the logarithms of the values for the analysis of variance. After transformation, the standard deviations were more nearly equal and uncorrelated with the means. The level of statistical significance selected was $P = 0.05$.

Table 1. *Expt* 1. *Radioactivity of the tissues of the wethers 26 h after administration of a single oral dose of* [^3H]DL-α-*tocopherol*

(In all experimental treatments, NMD-producing hay was given to four groups of five animals (weights 52–63 kg) for 1 year and was either unsupplemented (control), or supplemented weekly with an oral dose of selenium, or DL-α-tocopherol, or Se + DL-α-tocopherol. The amount of radioactivity given to each sheep was 4.93×10^7 dpm/kg body-weight. Values are mean logarithms of dpm/g fresh tissue with standard errors)

Tissue	No supplement	Se supplement	DL-α-Tocopherol supplement	DL-α-Tocopherol + Se supplement	SE
Liver	3·8270	3·6226	3·8267	3·9523	0·2162
Spleen	3·5996	3·5037	3·7173	3·3316	0·2249
Vena cava	3·2878	3·0794	3·3147	3·2690	0·1282
Abomasum	3·5446	3·6107	3·4917	3·5037	0·3765
Duodenum	3·2723	3·1046	3·3537	3·3712	0·2960
Adrenal	3·9858	3·7368	4·0076	3·8830	0·2047
Heart	3·0120	3·1879	2·9467	3·1855	0·1940
Lung	3·4830	3·4641	3·4709	3·3952	0·1800
Kidney	3·1678	3·1397	3·4856	3·3699	0·1393
Muscle	2·9865	2·7677	2·5174	2·7719	0·1749

NMD, nutritional muscular dystrophy; dpm, disintegrations/min.

The presence or absence of Se or tocopherol in the diet of the sheep during the 12 months before administration of radioactive tocopherol had no significant effect on the distribution of radioactivity in any of the tissues examined (Table 1). These results are in agreement with the findings of Diplock, Bunyan, McHale & Green (1967) and Green, Diplock, Bunyan, Muthy & McHale (1967) that Se does not affect the metabolism of tocopherol in rat tissues.

According to Cheeke & Oldfield (1969), plasma radioactivity after administration of tritiated vitamin E was lower in rats given a Se supplement than in untreated animals. Scott & Thompson (1968) found that Se increased the absorption and raised blood concentrations of vitamin E in chicks. On the other hand, Mukhtar (1966) reported that Se supplementation of diets for steers caused an increase in vitamin E in blood

Table 2. *Expt* 1. *Radioactivity of the blood of wethers after administration of a single dose of* [^3H]DL-α-*tocopherol*

(In all experimental treatments, NMD-producing hay was given to four groups of five sheep (weights 52–63 kg) for 1 year and was either unsupplemented (control), or supplemented weekly with an oral dose of selenium, or DL-α-tocopherol, or Se + DL-α-tocopherol. The amount of radioactivity given to each sheep was $4{\cdot}93 \times 10^7$ dpm/kg body-weight. Values are mean logarithms of dpm/g fresh blood with standard errors)

Sample time-interval (h)	No supplement	Se supplement	DL-α-Tocopherol supplement	DL-α-Tocopherol + Se supplement	SE
1	2·4799	2·8949	2·9194	2·8387	0·1142
2	2·8870	3·0129	3·1120	3·1496	0·1428
4	2·8986	3·2254	3·3348	3·1677	0·1103
6	2·8583	3·0053	2·9966	3·1343	0·1337
12	3·1095	3·3022	3·3917	3·2607	0·0994
24	3·0987	3·3472	3·3336	3·3847	0·1096

NMD, nutritional muscular dystrophy; dpm, disintegrations/min.

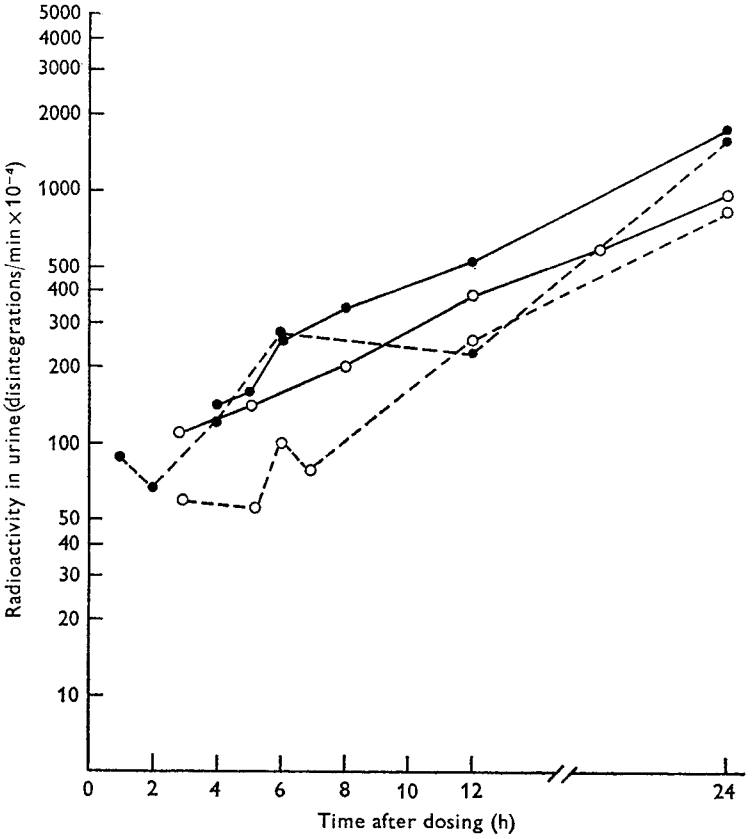

Fig. 1. Urine clearance of radioactivity after administration to sheep, intramuscularly or by stomach tube into the rumen, of a single dose of [^{3}H]DL-α-tocopherol. ●—●, sheep 27 ($8{\cdot}88 \times 10^8$ dpm, by stomach tube); ● – – ●, sheep 2 ($8{\cdot}88 \times 10^8$ dpm, intramuscularly); ○—○ sheep 48 ($1{\cdot}11 \times 10^9$ dpm, by stomach tube); ○ – – ○, sheep 73 ($1{\cdot}11 \times 10^9$ dpm, intramuscularly). dpm, disintegrations/min.

and a decrease in the liver. Mukhtar (1966) suggested that the reduction of vitamin E in the liver may be due to its transfer from liver to blood caused by Se functioning as a tocopherol carrier.

In our experiment, the presence or absence of Se or vitamin E, or of both, in the diet of sheep before administration of [^{3}H]tocopherol had no significant effect on the radioactivity recovered in blood at any of the sampling times (Table 2).

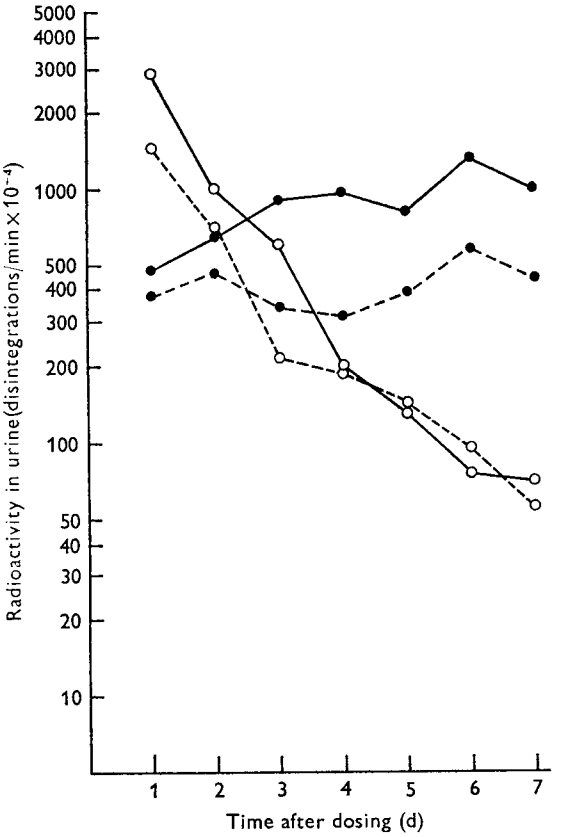

Fig. 2. Urine clearance of radioactivity after administration to sheep, intramuscularly or by stomach tube into the rumen, of a single dose of $1{\cdot}11 \times 10^9$ dpm [^{3}H]DL-α-tocopherol. ●—●, sheep 44 (intramuscularly); ● - - ●, sheep 26 (intramuscularly); ○—○, sheep 56 (by stomach tube); ○ - - ○, sheep 37 (by stomach tube). dpm, disintegrations/min.

From blood measurements taken immediately before slaughter (24 h after dosing) and from liver measurements at slaughter, regression analyses were calculated, using logarithmic transformations, to evaluate the possible relationships between these variables. The linear regression of dpm in blood on dpm in the liver ($r = -0{\cdot}407$) was not significant. In order to study the possibility that the relationship might be curvilinear, the analyses were again made after a second logarithmic transformation of the logarithms of dpm in the liver. The fit of the curve was not improved ($r = -0{\cdot}413$).

Expt 2. Maximum radioactivity in blood was recorded after 6 and 8 h for the intramuscular and intraruminal treatments respectively, followed by a slight decrease and

Table 3. *Expt 2. Radioactivity of fresh blood, and of the daily faeces and urine from wethers nos.* 70a, 55a, 83b and 88b given, by stomach tube into the rumen (IR) or intramuscularly (IM), a single dose of* $1{\cdot}11 \times 10^9$ *dpm* [^3H]*DL-α-tocopherol after having received NMD-producing hay for 9 months*

Time after dosing (d)	Total daily faecal excretion (dpm × 10^{-4})				Total daily urinary excretion (dpm × 10^{-4})				Blood activity (dpm × 10^{-2}/g)			
	70a IR	55a IM	83b IR	88b IM	70a IR	55a IM	83b IR	88b IM	70a IR	55a IM	83b IR	88b IM
1	64376	2522	59732	3800	1387	2416	1792	1285	84	397	38	421
2	26572	4142	23376	6738	1314	2819	507	1509	65	381	33	418
3	5688	3034	4495	5204	797	2949	204	1188	42	132	14	358
4	1188	2407	1394	3450	822	1827	90	824	60	234	20	239
5	351	2062	562	3073	133	1030	75	779	24	268	11	245
6	186	1518	—	2209	62	854	45	562	23	192	17	189
7	151	1440	441	1729	67	696	13	430	17	207	27	184
8	61	1229	330	1420	42	570	20	333	17	169	10	161
9	38	1157	250	1566	47	478	19	256	11	171	6	158
10	59	970	120	1560	38	447	28	202	—	165	10	138
11	53	843	51	1149	35	853	19	295	13	157	—	117
12	44	851	49	1309	34	582	18	293	12	175	11	109
13	30	1021	21	1223	33	515	22	308	10	160	7	122
14	11	522	8	653	26	774	15	249	—	102	9	111

NMD, nutritional muscular dystrophy; dpm, disintegrations/min.

* Sheep whose numbers are followed by the same letter were dosed at the same time: (*a*) 34–36 kg body-weight; (*b*) 37–38 kg body-weight.

Table 4. *Expt 2. Radioactivity of fresh tissues (dpm) and faeces (dpm × 10^{-4}) from wethers nos. 27, 2, 48 and 72, 24 h after a single dose of [^3H]DL-α-tocopherol administered orally by stomach tube into the rumen (IR) or intramuscularly (IM) after having received NMD-producing hay for 9 months*

	Tissue radioactivity				Sample time interval (h)	Faeces radioactivity			
Tissue	27 IR	2 IM	48 IR	73 IM		27 IR	2 IM	48 IR	73 IM
Liver	21044	36517	35921	61687	5	195	30	93	11
Adrenal	10594	21561	3555	20385	6	251	68	—	32
Spleen	4750	12811	4411	—	7	742	—	504	21
Lung	3353	9265	4091	7448	8	2099	195	1710	38
Vena cava	795	4356	—	1020	12	6226	—	14986	60
Kidney	2061	3106	4100	12342	24	58485	645	22658	1541
Pancreas	898	2003	1066	—	Total in 24 h	67998	948	39951	1703
Heart	961	2310	1520	2446					
Muscle	366	2001	2186	2395					
Abomasum	859	1623	1614	2059					
Duodenum	3671	1333	1220	4186					
Fat	742	2069	450	1578					
Testis	900	1434	1681	1072					

Sheep 27 and 2, at 37 kg body-weight, were dosed simultaneously with $8{\cdot}8 \times 10^8$ dpm; sheep 48 and 73, at 45 kg body-weight, were dosed simultaneously with $1{\cdot}11 \times 10^9$ dpm.

NMD, nutritional muscular dystrophy; dpm, disintegrations/min.

then an increase at 24 h. At these times and thereafter, blood radioactivity concentrations were much higher in sheep dosed intramuscularly than in those dosed intraruminally.

Intraruminal and intramuscular doses of [^{3}H]tocopherol were both rapidly excreted in the urine of sheep (Fig. 1). In the sheep dosed intraruminally, the urinary radioactivity values were highest at 24 h and thereafter declined sharply (Fig. 2). When sheep were injected intramuscularly, urinary radioactivity increased to a maximum in 3–6 d and subsequently declined steadily (Fig. 2, Table 3). These results show that in the sheep dosed intramuscularly the radioactivity of both blood and urine decreased much more slowly than in those dosed intraruminally (Table 3). This difference could be related to the slow release of [^{3}H]tocopherol from the site of injection into the circulatory system, if tocopherol introduced direct into the gastro-intestinal tract is eliminated rapidly. Radioactivity concentrations in the faeces indicate that sheep receiving tritium-labelled tocopherol intraruminally excreted the greater percentage of the dose during the first 3 d; in the intramuscularly injected sheep the excretion rate was much slower (Table 3).

There were significant differences (at the 1% level) in the metabolism of the tritiated vitamin E given by either method. One or more days after treatment, retention of radioactivity in the tissues was much greater in the sheep treated intramuscularly than in those dosed intraruminally (Tables 4 and 5). There was no substantial difference in the pattern of tissue distribution due to the mode of administration.

As reported previously (Hidiroglou, Jenkins & Carson, 1969), high uptakes of radioactivity were recorded at 24 h in some tissues, such as liver and adrenal, and lower uptakes in others, such as muscle (Table 5). However, at 2 weeks, these differences were less. Adipose tissue, testis and heart accumulated large amounts of radioactivity and they could be considered among the accumulator tissues. In these tissues, localization of tritiated vitamin E might be related to the physiological role of vitamin E. Rindi & Perri (1958) reported a significant increase in plasma tocopherol concentration in human beings after the parenteral administration of an aqueous solution of vitamin E. According to these authors, the ineffectiveness of intramuscular dosing reported by other investigators was due to the very slow absorption of the oil carrier which was used.

Our results are in agreement also with those of Johnson (1955) who reported that, following oral or intramuscular administration of [^{14}C]tocopherol, the percentage of the administered radioactivity recovered in the carcasses of rats 48 h after dosing was higher when the dose was given intramuscularly.

Differences in the ability of individual sheep to metabolize tocopherol, as reported in Expt 1, were found also in this experiment, presumably because there was variation in the rate of excretion between different sheep. The rate of excretion differed markedly with the two routes of administration of the radioactive tocopherol; vitamin E given orally in aqueous solution was excreted rapidly, which could explain the much lower residual radioactivity in the tissues of intraruminally treated than of intramuscularly treated sheep.

Table 5. *Expt 2. Effect of mode of administration, by stomach tube into the rumen (IR) or intramuscularly (IM) of [^3H]DL-α-tocopherol on the distribution of radioactivity in the tissue of wethers after having received NMD-producing hay for 9 months*

(Twelve sheep were divided into three groups of four. Groups 1 and 2 were given $8{\cdot}88 \times 10^8$ dpm and were killed after 7 and 14 d. Group 3 were given $2{\cdot}22 \times 10^9$ dpm and were killed at 10 d. In each group, two sheep were dosed by stomach tube and two intramuscularly. Sheep weights were 36, 32 and 42 kg for groups 1, 2 and 3 respectively. (*a*) Each value is the mean value for two sheep; (*b*) mean logarithms of the combined radioactivity values of fresh tissues determined at 7, 10 and 14 d, with a common value for the standard error)

	(*a*) Radioactivity of fresh tissue (dpm/g)						(*b*) Mean log of dpm/g fresh tissue		
	Group 1 at 7 d		Group 3 at 10 d		Group 2 at 14 d				
Tissue	IM	IR	IM	IR	IM	IR	IM	IR	SE
Liver	63230	3751	67410	3235	24600	2524	4·6536	3·4794	0·0838
Adrenal	53405	3351	70430	1783	35880	2747	4·7015	3·3592	0·0840
Spleen	23578	1507	24470	889	15095	968	4·3331	3·0295	0·0675
Lung	17470	2217	13746	652	16966	750	4·1956	2·9975	0·0869
Vena cava	10637	1592	5051	533	9250	1151	3·9530	2·9696	0·1048
Kidney	15630	2850	24158	1400	13270	967	4·2082	3·1780	0·0989
Pancreas	13688	2580	17927	1291	26350	2401	4·2660	3·2996	0·0591
Heart	18679	2408	15636	1735	19435	1452	4·2305	3·2194	0·0830
Muscle	3441	885	4733	481	4383	672	3·6827	2·8138	0·0446
Abomasum	5365	856	5734	419	7982	1273	3·7937	2·8783	0·0751
Duodenum	7364	732	7007	571	8125	1211	3·8536	2·8912	0·0693
Fat	10034	1346	18800	1511	23003	2301	4·2117	3·1975	0·0762
Testis	10182	1630	8807	627	11726	1283	3·9754	3·0190	0·0811

NMD, nutritional muscular dystrophy; dpm, disintegrations/min.

Table 6. *Expt* 3. *Mean logarithms of the plasma* DL-α-*tocopherol concentrations* (*μg*/*ml*) *of lambs from ewes that had received NMD-producing hay for* 3 *years*

(At 2 months of pregnancy twenty-three ewes (group B) were dosed at weekly intervals, by stomach tube into the rumen, with 1 g DL-α-tocopherol; the remaining nineteen ewes (group A) received no treatment. From birth to weaning eleven lambs from group B (B_1 lambs) and eleven from group A (A_1 lambs) were treated weekly with 500 mg vitamin E; the remaining sixteen lambs of group B (B_0 lambs) and thirteen of group A (A_0 lambs) were untreated)

Age (weeks)	All lambs					Lambs not given vitamin E (A_0 and B_0)			
	A_0	B_0	A_1	B_1	SD*	With severe NMD lesions	With very slight NMD lesions	Healthy	SE
1	0·0047 (13)	0·1316 (16)	1·0215 (11)	0·8645 (11)	0·21330	0·1060 (12)	0·0705 (10)	1·9988 (7)	0·20350
2	0·0033 (12)	0·0800 (14)	0·9904 (11)	0·7731 (11)	0·26168	$\bar{1}$·9415 (10)	0·0462 (8)	0·1719 (8)	0·25754
3	$\bar{1}$·9364 (12)	0·0497 (15)	0·6841 (11)	0·6556 (11)	0·30942	1·8403 (10)	0·0966 (9)	0·1085 (8)	0·35656
4	0·0084 (12)	$\bar{1}$·9880 (15)	0·7747 (11)	0·4995 (11)	0·29600	$\bar{1}$·8298 (10)	0·0960 (9)	0·0943 (8)	0·29852
5	$\bar{1}$·8492 (11)	$\bar{1}$·9575 (15)	0·7103 (11)	0·3941 (11)	0·33428	$\bar{1}$·6458 (9)	0·1064 (9)	$\bar{1}$·9914 (8)	0·32305
6	$\bar{1}$·8672 (11)	$\bar{1}$·8641 (13)	0·4982 (11)	0·3322 (9)	0·30010	$\bar{1}$·6729 (8)	0·0955 (8)	$\bar{1}$·8281 (8)	0·27749
7	$\bar{1}$·6388 (11)	$\bar{1}$·7268 (11)	0·3718 (10)	0·4559 (8)	0·31472	$\bar{1}$·5109 (7)	$\bar{1}$·9478 (7)	$\bar{1}$·6013 (8)	0·33175
8	$\bar{1}$·5419 (11)	$\bar{1}$·6684 (7)	0·4667 (10)	0·3011 (8)	0·32506	$\bar{1}$·2858 (5)	$\bar{1}$·7492 (5)	$\bar{1}$·6812 (8)	0·34416
9	$\bar{1}$·5550 (11)	$\bar{1}$·5934 (7)	0·2569 (9)	0·1977 (8)	0·24166	$\bar{1}$·3228 (5)	$\bar{1}$·6353 (5)	$\bar{1}$·6836 (8)	0·23586
10	$\bar{1}$·6729 (10)	$\bar{1}$·6459 (7)	0·3061 (9)	0·1128 (8)	0·18232	$\bar{1}$·6440 (4)	$\bar{1}$·6161 (5)	$\bar{1}$·7091 (8)	0·20247
11	$\bar{1}$·7046 (9)	$\bar{1}$·6110 (6)	0·1220 (6)	$\bar{1}$·9715 (8)	0·17055	$\bar{1}$·4717 (4)	$\bar{1}$·7403 (4)	$\bar{1}$·7371 (7)	0·11541
12	$\bar{1}$·5714 (8)	$\bar{1}$·6075 (4)	$\bar{1}$·9527 (6)	$\bar{1}$·9066 (8)	0·21723				
13	$\bar{1}$·5153 (8)	1·6981 (4)	$\bar{1}$·8894 (6)	$\bar{1}$·9774 (8)	0·22065				

* Pooled standard deviations within treatments. Figures in parentheses are the numbers of lambs. NMD, nutritional muscular dystrophy.

Expt 3. DL-α-Tocopherol given direct to lambs protected them completely from death due to NMD and elevation of serum aspartate aminotransferase. No such protection, however, was obtained by the untreated lambs born from ewes given 1 g DL-α-tocopherol weekly, six of which died from NMD. In this experiment, no vitamin E determinations were made on the milk of the ewes; however, an appreciable increase of tocopherol concentrations in the milk of cows following supplementation with vitamin E has been reported by Parrish (1949) and Merk & Crasemann (1961). On the other hand, Safford, Swingle & McRoberts (1956) found no correlation between NMD incidence and tocopherol concentrations in ewe's milk. The fact that NMD occurs mostly in the spring, when the young grass is rich in vitamin E, suggests that the tocopherol provided by ewe's milk may be biologically unavailable to their lambs. This is supported by the present results, in which plasma vitamin E concentrations in the unsupplemented lambs from either treated or untreated ewes were of the same magnitude (Table 6). It is possible that either the availability to the lambs of vitamin E from ewe's milk was impaired by the presence of an antagonistic substance or its retention was greatly diminished by the absence of some dietary component not present in the milk.

The concentrations of vitamin E in the plasma were much higher in lambs dosed with vitamin E than in those untreated (Table 6). Although this difference was initially large, it diminished thereafter, in spite of the fact that the treated lambs continued to receive 500 mg vitamin E weekly. Such decrease of plasma tocopherol concentrations with age could be related either to the diminishing capacity of a component of the plasma or to some defect in the absorption by the lambs of this lipid-soluble vitamin. The variability in the vitamin E concentrations of the plasma was great and was more pronounced in the vitamin E-treated lambs.

As reported previously, none of the vitamin E-treated lambs died from NMD or showed SGOT values of more than 200 Frankel Units. The percentages of untreated lambs with elevated serum aspartate aminotransferase values (> 200) at 1, 3, 5, 7 and 9 weeks of age were 33, 66, 50, 45 and 40 for the lambs born from the untreated ewes, and 15, 36, 37, 33 and 33 for those born from vitamin E-supplemented ewes.

According to Culik, Bacigalupo, Thorp, Luecke & Nelson (1951), NMD in lambs is characterized by low tocopherol concentrations in blood, with critical values below 0·081 mg of total tocopherol/100 ml of blood. In our experiment, death from NMD was associated with such low plasma tocopherol concentrations, and similarly low tocopherol concentrations were recorded in the plasma of untreated lambs that were healthy (Table 6) but had high serum aspartate aminotransferase values.

All the results obtained in this experiment, including those for serum tocopherol concentrations, clinical and post-mortem examinations and serum aspartate aminotransferase determinations, confirm the findings of Hogue, Proctor, Warner & Loosli (1962) that α-tocopherol is utilized for the prevention of NMD when given direct to the lambs but not when given to the ewes.

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