Vitamin E kinetics in sheep

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1. Kinetics of physiological doses of D-α-[5-Me-^{3}H]tocopherol (200 μCi) administered to twenty-four sheep were studied using one of four routes: intravenous, oral (capsules), intraruminal and intramuscular.

2. Blood samples were withdrawn from the jugular vein periodically for 96 h after the intravenous and oral administrations, for 168 h after the intraruminal administration and for 216 h after the intramuscular administration.

3. The study indicated that the biological availability of α-tocopherol followed the order intravenous > intramuscular > oral > intraruminal.

4. The rate of elimination was in the order intravenous > oral > intraruminal ~ intramuscular.

5. The intravenous route was fitted with a three-compartment model, whereas the other routes exhibited a good fit for either a one- or two-compartment model.

The importance of vitamin E in the prevention of nutritional muscular dystrophy in young ruminants led to investigations in our laboratory concerned either directly or indirectly with the metabolism of vitamin E in sheep (Hidiroglou *et al.* 1970; Hidiroglou & Jenkins, 1974; Hidiroglou, 1977). Sternberg & Pascoe-Dawson (1959) introduced the use of labelled α-tocopherol in order to estimate the absorption and turnover of this vitamin following oral administration to rats. According to Weber (1983), a selection of variables measuring α-tocopherol absorption is of significance. Thus the mode of administration of α-tocopherol is of considerable influence on the absorption of vitamin E. There is, however, a paucity of information on compartment volumes and exchange rates of α-tocopherol in the biological system of domestic animals. The purpose of the present work was to study and compare α-tocopherol kinetics in sheep, following administration of D-α-[5-Me-^{3}H]tocopherol by various routes.

EXPERIMENTAL

Animals

Yearling crossbred wethers, weighing 45–48 kg, were used. All animals originated from a flock born and raised in confinement. The animals were fed, for 6 months before and during the experiment, on a diet which consisted of (g/kg) grass silage 400, hay 400, maize silage 200.

The sheep were placed in metabolism cages 10 d before dosing. During this period blood samples were obtained daily (08·00 hours) from the jugular vein by venepuncture for baseline values. A total of twenty-four sheep were used to study four routes of administration: intravenous, oral, intraruminal and intramuscular. For each route of administration six sheep were each dosed with 200 μCi ^{3}H-labelled D-α-tocopherol in 2 ml ethanol without any carrier. Intravenous and intramuscular doses were injected into the jugular vein and gluteal muscle respectively. Oral doses were contained in capsules and administered

by an ovine balling-gun. For intraruminal administration, the dose was injected through the left flank directly into the rumen, using an 80 mm long hypodermic needle. Precautions were taken to ensure that the tracer was introduced into the rumen and not into the peritoneal cavity.

Blood samples (10 ml) from the jugular vein were taken at frequent intervals after morning (08.30 hours) dosing. Samples were collected in heparinized tubes and centrifuged. The plasma was removed and stored at $-20°$ until assayed for radioactivity and D-α-tocopherol. The blood sampling period was 96 h for the intravenous and oral doses, 168 h for the intraruminal dose and 216 h for the intramuscular dose. After completion of the sampling period the sheep were killed by intravenous injection of sodium pentobarbitol.

Materials

Standard D-α-tocopherol was purchased from Eastman–Kodak (Rochester, New York) and D-α-[5-Me-^{3}H]tocopherol with a specific activity of 24 Ci/mmol or 55·7 mCi/mg was purchased from Amersham International plc, Amersham, Bucks. Its purity was not less than 95%, as determined by thin-layer chromatography. Standards D-α, D-γ and D-δ-tocopherol were purchased from Eastman–Kodak.

Analysis of radioactivity

Triplicate samples (0·5 ml portions) of plasma were assayed for total radioactivity in 10 ml of a phase-combined system (Aquasol 2-scintillation fluid, New England Nuclear, Boston, Massachusetts). All samples were counted in a Beckman LS 280 liquid scintillation spectrometer (Beckman Instruments Ltd, Fullerton, California) using an automatic external standard. The counting efficiency of ^{3}H was 40 (SD 3)% and the counts were corrected for background and quenching effects. All results were converted into disintegrations/min (dpm).

Analytical

High-performance liquid chromatography (HPLC) with a fluorescent detector was used for plasma D-α-tocopherol determination (McMurray & Blanchflower, 1979*b*). The HPLC system consisted of an M 6000 pump and WK septumless injector (Waters Associates Inc., Milford, Massachusetts). A Perkin-Elmer 650-105 fluorescence spectrophotometer, equipped with a microflow-cell unit, was used for quantitative determinations. Wavelength settings were 295 and 330 nm for excitation and emission respectively. The column was a μ Bondapak C_{18} (3·9 × 300 mm) of 10 μm particle size purchased from Waters Associates Inc. Elution was performed with methanol–water (97:3, v/v) solvent with a flow rate of 3 min/ml. All solvents used for the mobile phase of HPLC or for the extraction were HPLC or pesticide grade. Blood plasma (1 ml) samples were precipitated with ethanol (1 ml) and extracted with hexane (1 ml). Identification and quantitative determination of α-, β-, γ- and δ-tocopherols were by comparison of retention times and peak areas with tocopherol standards. Tocopherol determination in roughage was performed according to the technique of McMurray & Blanchflower (1979*a*).

Statistical methods

Characterization of the D-α-[^{3}H]tocopherol specific activity profiles was based on non-linear least squares applied to one, two- and three-compartment pharmacokinetic models. For estimation purposes the models were represented as sums of exponentials. Intravenous dose profiles were represented in the form:

$$\log c(t) = \log [\Sigma A_i \, e^{-k_i t}] + e(t),$$

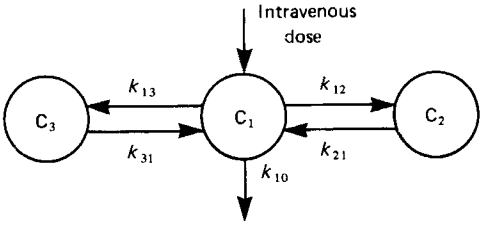

Fig. 1. Kinetics of D-α-[^{3}H]tocopherol described as a three-compartment exchange system in sheep. C_1, central compartment; C_2, shallow compartment; C_3, peripheral compartment; k_{12}, k_{21}, k_{13}, k_{31}, intercompartmental transfer rates; k_{10}, elimination rate.

where $c(t)$ denotes the specific activity of D-α-[^{3}H]tocopherol at time t, $e(t)$ is a random error term which is assumed to be normally and independently distributed with mean 0 and variance σ^2, and A_i, k_i are parameters to be estimated. For oral, intraruminal and intramuscular doses, plasma profiles generally consisted of an initial period of rapid increase in specific activity followed by an apparent first-order absorption and elimination period. Hence these profiles were represented in the form

$$\log c(t) = \log[A_i(1-e^{-k_i t})]+e(t), \quad t < T,$$
$$= \log[\sum_{i=2} A_i e^{-k_i t}]+e(t), \quad t \geqslant T,$$

where T represents the transition point between the initial rapid-increase phase and the first-order phase. For each plasma profile the model described previously was fitted repeatedly using each of the sampling times as a transition point. The optimal transition point was determined for each profile by using a minimum pooled residual mean-squared error criterion (Hudson, 1966). The corresponding estimated exponential equation was taken as the optimal representation of the profile.

As indicated in the previously described models, analyses were carried out on the logarithmic scale. This transformation was required in order to stabilize variances which, according to preliminary analyses, were approximately proportional to the observed concentrations. Parameter estimation was carried out using the SAS non-linear regression procedure (SAS Institute, 1982) with the Marquardt iterative method. For the intravenous doses, two- and three-compartment models were fitted. For the first-order absorption and elimination phase of the oral, intraruminal and intramuscular dose profiles, one- and two-compartment models with first-order absorption were considered. In each case attempts were made at fitting the higher-order model. For all cases where a higher-order model could be estimated, the adequacy of the lower-order model was verified through examination of the least-squares residuals and goodness-of-fit F tests.

In order to obtain some additional understanding of the transfer and elimination of α-tocopherol, a commonly used compartmental modelling approach (Ramsey & Andersen, 1984) was employed. Our intravenous dose data are consistent with the three-compartment configuration shown in Fig. 1. C_1 is the central compartment which consists of the blood and all readily accessible fluids and tissues. The shallow compartment, C_2, could be assumed to consist of a grouping of highly perfused tissues and organs. The less perfused regions are represented by the deep peripheral compartment, C_3. It was further assumed that elimination occurs only from the central compartment, C_1. Validation of the physiological appropriateness of this mathematical model could not be carried out at the present time. Our data did not allow physiological modelling since information was not available for the various intrinsic volumes, blood flow rates, partition coefficients and biochemical constants specific to the chemical under investigation.

Table 1. *Exponential equation parameter estimates for profiles of the* D-α-[^3H]*tocopherol specific activity* ($c(t)$) *and average* D-α-*tocopherol levels in the plasma after intravenous injection*

(Mean values and standard deviations of the estimates)

Sheep no.	$c(t) = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} + A_3 e^{-k_3 t}$						D-α-tocopherol (μg/ml)
	Intercept (dpm per μg)			Exponent (/h)			
	A_1	A_2	A_3	k_1	k_2	k_3	
1 Mean	109213	32821	14214	39·4391	0·2696	0·0118	0·77
SD	24303	2040	938	9·2807	0·0267	0·0011	0·01
2 Mean	38621	29974	12134	16·2091	0·4971	0·0147	0·79
SD	11498	4031	1008	8·5075	0·941	0·0014	0·01
3 Mean	75653	42391	8040	21·1976	0·2924	0·0082	0·74
SD	18678	3362	741	7·2543	0·0275	0·0015	0·02
4 Mean	395932	28194	12712	54·0869	0·9658	0·0132	0·72
SD	109275	3835	1051	10·9726	0·2471	0·0015	0·01
5 Mean	124599	40399	16892	13·3254	0·3759	0·0203	0·74
SD	29390	7802	2573	4·7529	0·0963	0·0028	0·01
6 Mean	2058599	52494	17220	72·5160	0·4557	0·0145	0·75
SD	591336	6750	2089	11·1225	0·0948	0·0020	0·01

t, Time; dpm, disintegrations/min.

Based on the estimated parameters in the multiexponential equations, derived parameters for the intravenous dose profiles were calculated (Gibaldi & Perrier, 1975) using the three-compartment configuration shown in Fig. 1. Estimates were also derived for the half-life ($t_{\frac{1}{2}}$) of the terminal elimination phase, $t_{\frac{1}{2}} = 0{\cdot}693/k_3$, the volume of the central compartment, (V_c),

$$V_c = \frac{\text{dose}}{\sum_1 A_i} \Big/ \alpha\text{-tocopherol level},$$

the transport rate (TR),

$$TR = (CR) \times (\alpha\text{-tocopherol level})$$

and the clearance rate (CR),

$$CR = k_{10} V_c.$$

For these calculations dose was expressed in dpm (i.e. 200 μCi = $200 \times 2{\cdot}2 \times 10^6$ dpm). Specific activity and α-tocopherol levels were measured in dpm/μg and μg/ml respectively. Thus V_c, TR and CR were expressed in terms of ml, μg/h and ml/h respectively.

Areas under the specific activity profiles were calculated as integrals of the estimated multiexponential equations up to 168 h. For oral and intraruminal doses, where an insufficient number of observations were available to provide a proper estimate of the equation for the initial rapid increase, the trapezoidal rule was used to calculate this portion of the area.

Inter-route comparisons for various parameters were based on analysis of variance F tests and on t tests using a pooled residual mean-squared error.

In all analyses aberrant observations which were judged to be incompatible with the remaining data were excluded from statistical analyses. These were observations which, on the basis of tests of residuals, were identified as probable outliers (Grubbs, 1969) and had a disproportionate adverse influence on parameter estimates (Belsley *et al.* 1980).

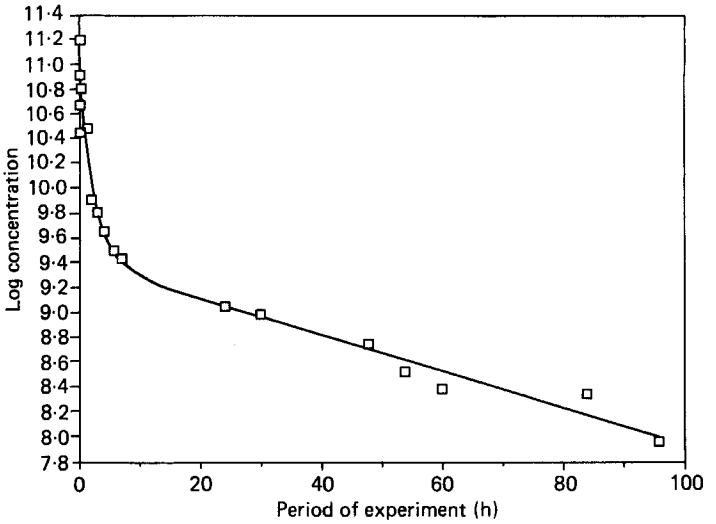

Fig. 2. Profiles of observed and predicted log plasma concentrations of D-α-[^{3}H]tocopherol after intravenous injection: sheep no. 2. □, Observed; ——, predicted.

RESULTS

Tocopherol contents of roughages

The averages of six determinations of tocopherol contents of roughage at the time of consumption were respectively 39, 64 and 32 mg/kg dry matter for maize silage, grass silage and hay. Only α-tocopherol was found in hay and grass silage. In maize silage α-tocopherol comprised, on average, about half the total tocopherol and was equal to the sum of α- + γ- (26%) and δ-(24%) tocopherols.

Animals

Intravenous route. In each case the D-α-[^{3}H]tocopherol profile in the plasma was best represented as a sum of three exponentials:

$$c(t) = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} + A_3 e^{-k_3 t}.$$

Parameter estimates for each animal are presented in Table 1. Average D-α-tocopherol levels in the plasma over the blood sampling period are also presented in Table 1.

The first phase of the profile (i.e. $A_1 e^{-k_1 t}$) appears to be a distributive phase which is clearly evident only during the first 5 min (Fig. 2). Beyond this point the decay profile is dominated by the second and third exponential terms (i.e. $A_2 e^{-k_2 t} + A_3 e^{-k_3 t} > A_1 e^{-k_1 t}$).

The second phase (i.e. $A_2 e^{-k_2 t}$) of the profile is clearly evident up to 6 h in some sheep (i.e. $A_2 e^{-k_2 t} > A_3 e^{-k_3 t}$). Beyond 20 h all profiles were essentially in the terminal decay phase represented by $A_3 e^{-k_3 t}$.

Based on the estimates in Table 1 and the three-compartment configuration given in the statistical methodology section, parameter estimates were derived for the intercompartmental transfer rates $k_{12}, k_{21}, k_{13}, k_{31}$; the elimination rate k_{10}; the $t_{\frac{1}{2}}$ of the terminal elimination phase; the volume of the central compartment; the transport rate; and the clearance rate. These estimates are provided in Table 2.

Oral routes. After oral administration of α-tocopherol, no detectable specific activity level was observed in the plasma at 0·17 h. By the time of the first measurable appearance of labelled tocopherol, the specific activity levels were consistently higher than would be

Table 2. *Kinetic parameter estimates* for the three-compartment model of* D-α-[^3H]-*tocopherol specific activity in the plasma after intravenous injection*

Sheep no.	Elimination rate, k_{10} (/h)	Intercompartmental transfer rate (/h)				Volume of the central compartment (l)	Terminal elimination phase half-life (/h)	Transport rate (μg/h)	Clearance rate (ml/h)
		k_{12}	k_{21}	k_{13}	k_{31}				
1	0·117	26·990	12·007	0·518	0·089	3·66	58·8	331·1	429·9
2	0·091	7·259	8·629	0·592	0·151	6·90	47·2	495·4	627·1
3	0·112	12·207	8·628	0·499	0·053	4·72	84·5	389·7	526·6
4	0·438	42·141	5·704	6·506	0·277	1·40	52·4	440·2	611·4
5	0·192	8·365	4·387	0·657	0·121	3·27	34·1	463·7	626·6
6	1·602	60·241	2·724	8·310	0·110	0·28	47·7	330·5	440·7

* Estimates are based on the three-compartment configuration given in Fig. 1.

expected with a first-order absorption process. There was a short time-period, up to time T say, during which specific activity levels increased quite rapidly, similar to the rapid increase that would be observed with an intravenous infusion process. Since there were insufficient data to allow elucidation upon the absorption, distribution and elimination characteristics in this short time-period, the intravenous infusion model

$$c(t) = A_1(1-e^{-k_1 t}), \quad t < T \tag{M1}$$

was used for this initial portion of the specific activity profile. After the initial rapid increase the profile was well represented as a first-order process. Assuming a one-compartment model with first-order absorption and elimination after time T, the remainder of the profile would be represented by

$$c(t) = A_1(1-e^{-k_1 T})e^{-k_2(t-T)} + A_2^*(e^{-k_2 t} - e^{-k_3 t}), \quad t \geqslant T.$$

The first component of this equation represents the first-order elimination of the levels accumulated to time T. The second component represents the standard one-compartment first-order absorption and elimination. With the obvious re-parameterization the equation reduces to

$$c(t) = A_2 e^{-k_2 t} - A_3 e^{-k_3 t}, \quad t \geqslant T. \tag{M2}$$

With a two-compartment model the corresponding equation would reduce to

$$c(t) = A_2 e^{-k_2 t} + A_3 e^{-k_3 t} - A_4 e^{-k_4 t}, \quad t \geqslant T. \tag{M3}$$

In fitting these equations to the oral administration profiles, allowance was also made for a possible lag time. Optimal estimates of the transition point T between the initial rapid increase and the first-order phase were obtained as described in the statistical methods. Resulting estimates of T and the parameter estimates for eqn (M2) over the period from T to 96 h are provided in Table 3. All profiles were consistent with a one-compartment model with first-order absorption after 1 h (Fig. 3). The two-compartment eqn (M3) could not be estimated for all six sheep. No estimate of eqn (M1) for the profiles over time-periods from 0 h to T h is provided, since there were insufficient samples at times before T h.

Intraruminal and intramuscular routes. For the intraruminal and intramuscular administrations, the first post-administration sampling times were 0·33 and 0·25 h respectively. Since specific activity levels were always positive at these time points, there was no suggestion of a lag time such as was observed at earlier sampling times in the oral

Table 3. *Exponential equation parameter estimates for profiles of the* D-α-[^3H]*tocopherol specific activity* ($c(t)$) *and average* D-α-*tocopherol levels in the plasma after oral administration*

(Mean values and standard deviations of the estimates)

			$c(t) = A_2 e^{-k_2 t} - A_3 e^{-k_3 t}$				
			Intercept (dpm per μg)		Exponent (/h)		
Sheep no.	Time interval* (h)		A_2	A_3	k_2	k_3	D-α-tocopherol (μg/ml)
7	1–96	Mean	6554	5323	0·0115	0·0888	0·71
		SD	1411	1342	0·0030	0·0290	0·01
8	0·33–72	Mean	3116	2596	0·0083	0·2049	0·72
		SD	300	289	0·0020	0·0364	0·01
9	0·17–96	Mean	2553	2424	0·0050	0·2382	0·72
		SD	195	191	0·0012	0·0316	0·01
10	1–96	Mean	2780	2618	0·0093	0·2873	0·72
		SD	203	187	0·0012	0·0561	0·01
11	0·1–96	Mean	2382	2135	0·0071	0·2577	0·76
		SD	198	194	0·0014	0·0409	0·01
12	0·05–96	Mean	2443	2388	0·0070	0·2648	0·79
		SD	215	213	0·0015	0·0364	0·01

t, Time; dpm, disintegrations/min.
* The time interval specifies the observation period over which the equation was fitted.

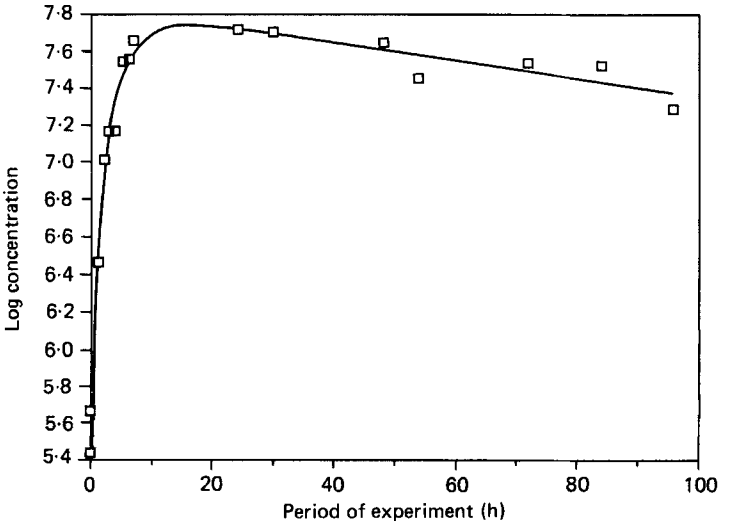

Fig. 3. Profiles of observed and predicted log plasma concentrations of D-α-[^{3}H]tocopherol after oral administration: sheep no. 9. □, Observed; ——, predicted.

Table 4. *Exponential equation parameter estimates for profiles of the* D-α-[^3H]*tocopherol specific activity* ($c(t)$) *and average* D-α-*tocopherol levels in the plasma after administration directly into the rumen via a syringe*

(Mean values and standard deviations of the estimates)

			$c(t) = A_2 e^{-k_2 t} + A_3 e^{-k_3 t} - A_4 e^{-k_4 t}$						
			Intercept (dpm per μg)			Exponent (/h)			
Sheep no.	Time interval (h)		A_2	A_3	A_4	k_2	k_3	k_4	D-α-tocopherol (μg/ml)
13	1–168	Mean	3720	1265	4713	0·0523	0·0012	0·1130	0·64
		SD	7228	327	7515	0·0485	0·0018	0·0687	0·01
14*	1–168	Mean	1738	—	1330	0·0054	—	0·2155	0·61
		SD	64	—	71	0·0004	—	0·0271	0·01
15	1·33–168	Mean	1360	1684	2617	0·0337	0·0038	0·1542	0·55
		SD	634	607	980	0·0346	0·0022	0·0480	0·01
16	1·67–168	Mean	1346	584	1600	0·0190	0·0000	0·1354	0·79
		SD	912	1494	726	0·0328	0·0122	0·0626	0·01
17*	0·33–168	Mean	1906	—	1821	0·0061	—	0·3231	0·56
		SD	139	—	132	0·0008	—	0·0540	0·01
18*	0·67–144	Mean	2448	—	1983	0·0056	—	0·2056	0·62
		SD	211	—	206	0·0010	—	0·0473	0·01

t, Time; dpm, disintegrations/min.

* The time-interval specifies the observation period over which the equation was fitted. Profiles for sheep nos. 14, 17 and 18 were properly represented with only one exponential elimination phase.

Table 5. *Exponential equation parameter estimates for profiles of the* D-α-[^3H]*tocopherol specific activity* ($c(t)$) *and average* D-α-*tocopherol levels in the plasma after intramuscular injection*

(Mean values and standard deviations of the estimates)

	Rapid absorption phase $c(t) = A_1(1 - e^{-k_1 t})$				Slower absorption phase and elimination phase $c(t) = A_2 e^{-k_2 t} - A_3 e^{-k_3 t}$					
			Intercept (dpm per μg)	Exponent (/h)		Intercept (dpm per μg)		Exponent (/h)		
Sheep no.	Time interval* (h)		A_1	k_1	Time interval* (h)	A_2	A_3	k_2	k_3	D-α-tocopherol (μg/ml)
19	0–3	Mean	1245	1·841	4–216	2848	3779	0·0034	0·2511	0·96
		SD	45	0·167		107	1031	0·0003	0·0630	0·02
20	0–1·75	Mean	1519	1·928	4–216	4557	3009	0·0033	0·0772	0·69
		SD	69	0·198		229	223	0·0003	0·0169	0·01
21	0–1·0	Mean	1737	2·326	1·25–216	4347	3037	0·0033	0·1547	0·71
		SD	125	0·330		137	159	0·0002	0·0198	0·03
22†	—		—	—	—	—	—	—	—	0·46
										0·02
23	0–1·75	Mean	1722	1·724	2–216	4925	4659	0·0057	0·1456	0·79
		SD	158	0·335		339	444	0·0005	0·0311	0·02
24	0–4	Mean	2332	0·811	5–216	6734	4260	0·0051	0·0232	0·76
		SD	136	0·081		2316	2215	0·0016	0·0115	0·01

t, Time; dpm, disintegrations/min.

* The time interval specifies the observation period over which the equation was fitted.

† Sheep no. 22 had abnormally low α-tocopherol levels and hence the specific activity profile was not fitted.

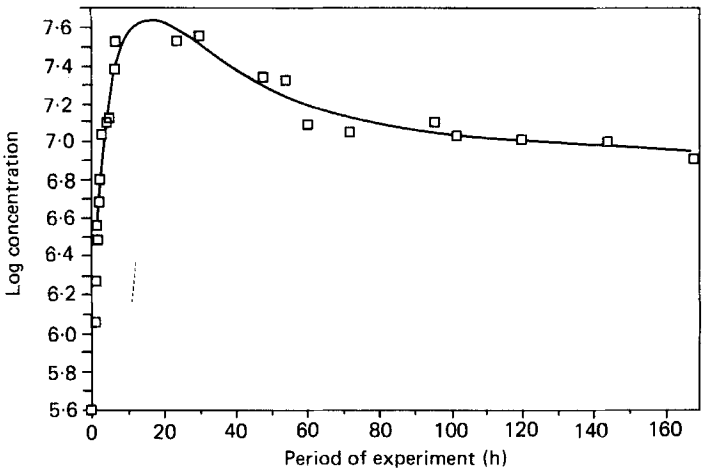

Fig. 4. Profiles of observed and predicted log plasma concentrations of D-α-[^{3}H]tocopherol after intraruminal administration: sheep no. 13. □, Observed; ——, predicted.

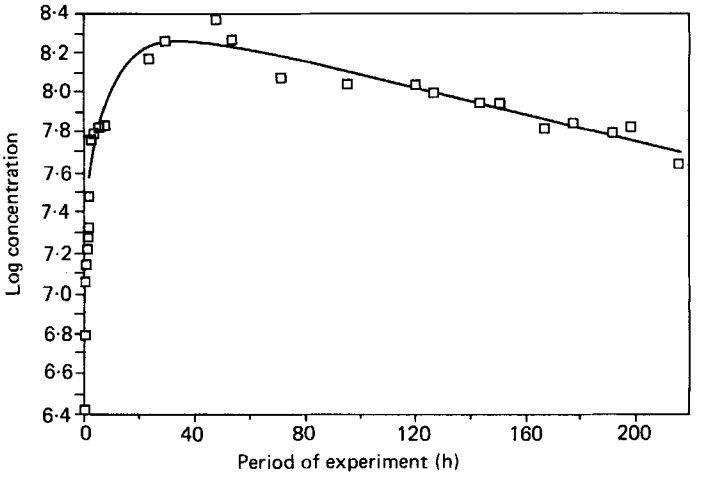

Fig. 5. Profiles of observed and predicted log plasma concentrations of D-α-[^{3}H]tocopherol after intramuscular administration: sheep no. 20. □, Observed; ——, predicted.

administrations. However, the intraruminal and intramuscular profiles did exhibit the biphasic structure and hence the estimation procedure described previously for the oral profiles was also applied here. Parameter estimates are provided in Tables 4 and 5.

As with the oral profiles, there were insufficient values after intraruminal administration to model the initial rapid increase from 0 to T h. Beyond T h the intraruminal profiles did not reflect a consistent degree of compartmentalization. Profiles for sheep nos. 13, 15 and 16 were best fitted by eqn (M 3) (Fig. 4), indicating a two-compartment system, while good fits of the profiles for sheep nos. 14, 17 and 18 were obtained with the one-compartment system represented by eqn (M 2).

For the intramuscular profiles, a prolonged period of rapid increase was observed, and estimates of eqn (M 1) for the period from 0 to T h are also provided in Table 5. Profiles from T to 216 h were all consistent with eqn (M 2) (Fig. 5). Sheep no. 22 was excluded from all analyses since it had abnormally low D-α-tocopherol levels.

Table 6. *Averages for profile summary statistics by route of administration*
(Mean values and coefficients of variation (CV); 95% confidence limits in parentheses)

		Area under curve from 0 to 168 h ((dpm/μg) × h)	Relative availability (%)	Specific activity 60 h:168 h	Peak specific activity (dpm/μg)	Time to peak specific activity (h)	Specific activity at 1 h (dpm/μg)
Intravenous	Mean	973113	—	4·43	—	—	—
	CV (%)	0·16		0·45			
Oral	Mean	269502	27·7 (21·8, 35·2)	2·40	2567	15·9	882
	CV (%)	0·24		0·24	0·27	0·26	0·37
Intraruminal	Mean	204689	21·0 (16·5, 26·8)	1·67	1822	16·1	369
	CV (%)	0·18		0·27	0·19	0·12	0·20
Intramuscular	Mean	496215	51·0 (39·6, 65·7)	1·50	3655	31·9	1021
	CV (%)	0·20		0·12	0·18	0·51	0·24

dpm, Disintegrations/min.

Effects of route of administration. A number of values relevant in comparing profiles across routes of administration are provided in Table 6.

The availabilities of tocopherol following administration via the oral, intraruminal and intramuscular routes relative to the intravenous route were very low. The intraruminal route produced significantly lower availability than the oral route ($P = 0{\cdot}027$). The intramuscular route produced a significantly higher ($P < 0{\cdot}001$) relative availability than either the oral or intraruminal route.

Comparison of elimination characteristics was complicated by the fact that duration of blood sampling was not consistent across routes and the fitted exponential models did not reflect consistency in the elimination kinetics. Hence a comparison of elimination rates was based on the specific activities ratio at two separate time-points in the elimination phase, namely 60 h and 168 h. The time-point 60 h was chosen as one of the earliest times which could be regarded as beyond the absorption phase and 168 h was a compromise between the actual terminal sampling times for the four routes of administration.

Elimination rates were very similar for the intraruminal and intramuscular routes. For the oral route elimination appeared to be significantly ($P < 0{\cdot}04$) more rapid. The intravenous elimination rates were substantially ($P < 0{\cdot}001$) faster than those in the other three routes.

DISCUSSION

In the present investigation a multicompartmental method of analysis has been used to measure tracer kinetics. An important assumption in the present study is that the system is in a steady state. That is, the pool of α-tocopherol and flow-rates are not affected by the administration of D-α-[^{3}H]tocopherol with a high specific activity. Results of the α-tocopherol levels (Tables 1, 3–5) over the study period was small and no systematic trend was observed.

A further concern is the extent to which the tracer reflects D-α-tocopherol kinetics. In the present study, the radioactivity following intravenous administration was identified almost exclusively as unchanged α-tocopherol. This result is in agreement with previously reported studies. Hidiroglou (1977) reported that over 90% of the radioactivity in the plasma was as α-tocopherol in a study with sheep following dosage of D-α-[^{3}H]tocopherol.

Csallany *et al.* (1971) had dosed rats intravenously with D-α-[^{14}C]tocopherol and reported that all the radioactivity in plasma and erythrocytes was present as α-[^{14}C]tocopherol. Krishnamurthy & Bieri (1963) and Mellors & Barnes (1966) reported tissue and blood radioactivities as entirely unchanged D-α-tocopherol following oral administrations of D-α-[^{14}C]tocopherol in rats. Gallo-Torres (1970) reported lymph radioactivity following oral doses of an emulsion of radioactive DL-α-tocopherol acetate as mostly unesterified D-α-tocopherol with only minor amounts as DL-tocopherol ester. We therefore concluded that measurement of D-α-[^{3}H]tocopherol could be validly used to study the kinetics of unchanged D-α-tocopherol.

Profiles of D-α-[^{3}H]tocopherol in the plasma following intravenous injection were best represented as a sum of three exponentials (Fig. 2). For each sheep a significant ($P < 0{\cdot}01$) improvement in fit was found over the two-exponential model. The first phase consisted of an extremely rapid decline in specific activity, which was probably due to an exchange in labelled tocopherol between the plasma and extracellular fluids. The rapidity of this exchange was reflected in the magnitude of k_{12} (Table 2), the transfer rate from compartment 1 to compartment 2. Since this phase was clearly evident only in the first 5 min after administration, estimates of the exponential parameters (A_1 and k_1) were not very precise, as evidenced by the large standard deviations in Table 1. Nevertheless, observation of this early phase was important in the study of D-α-tocopherol kinetics since estimates of pharmacokinetic parameters (e.g. k_{ij} and V_c in Table 2) could be substantially biased if this phase were ignored. Identification of the relevant compartments involved would require further experimentation. It is also possible that higher-order models may be appropriate if the D-α-[^{3}H]tocopherol profile were observed over a longer period. While a four-exponential model could not be estimated with the present values, it is noted that plasma sampling was terminated at 96 h when specific activity levels were still relatively high (geometric mean of 3849 dpm/μg at 96 h).

Comparison of the D-α-[^{3}H]tocopherol profiles for the oral, intraruminal and intramuscular administrations was complicated by a number of factors. The oral route appeared to have a lag time before measurable concentrations were found. All three routes of administration exhibited a biphasic absorption profile. For the intraruminal administration three of the elimination profiles were adequately represented by a single exponential while the remaining three required a sum of two exponentials.

The lag time observed for the oral administration was not unexpected and was at least partially due to the time required for the capsules to dissolve. Another explanation for the lag time was suggested by the rapid decline in D-α-[^{3}H]tocopherol in the first 5 min after administration of the intravenous doses. A rapid exchange in labelled tocopherol between plasma and extracellular fluids could result in plasma levels below detection limits within the first few minutes after oral administration.

The biphasic absorption profile for the oral, intraruminal and intramuscular routes consisted of a short period of rapid increase in specific activity followed by a period of apparent first-order absorption. The concentration profile during the period of rapid increase was comparable to the profile that might be observed during an infusion process. This rapid increase might be due to saturation at the site of administration with a consequent spill-over into the plasma.

The intraruminal route produced a significantly lower availability than either the oral ($P \leqslant 0{\cdot}027$) or the intramuscular ($P \leqslant 0{\cdot}001$) route. The difference between the oral and intraruminal routes appeared to be due mainly to the large specific activities for the oral profiles in the early post-administered period. Although both routes had initial rapid increases of similar duration, the increase was more rapid after oral administration. At 1 h the oral specific activities were 1·5 times ($P < 0{\cdot}001$) as high as the intraruminal specific

activities and the increased levels were maintained throughout the absorption period. Intramuscular relative biological availabilities were significantly higher ($P < 0{\cdot}001$) than for either the oral or intraruminal routes. At the 1 h sampling time specific activities for the intramuscular route were already 1·6 and 2·3 times higher than the oral and intraruminal levels respectively. Also, the initial period of rapid increase lasted longer and absorption continued for a longer period of time. The average time to peak specific activity was 31·9 h for the intramuscular route compared with 16 h for the oral and intraruminal routes. According to MacMahon *et al.* (1971) the lymphatic pathway would be the main route of absorption for the oral and intraruminal routes. The reduced level of absorption with these two routes appears to reflect a reduced transfer of D-α-tocopherol through the intestinal lumen.

The duration of absorption following oral doses appeared to be slightly longer than those reported by other authors for other species. In human studies Kelleher & Losowsky (1970) reported peak times for plasma radioactivity ranging from 6 to 12 h while Blomstrand & Forsgren (1968) reported peak times of 4–6 h. Poukka & Bieri (1970) reported peak specific activities in both plasma and cells at 8 h after oral administration of D-α-[^{3}H]tocopherol to rats. The single exponential decay curves representing the elimination phase were similar to those reported by Silber *et al.* (1969) in rats following oral doses of radiolabelled D-α-tocopherol.

Apparent differences in elimination profiles between the oral and intraruminal routes did not necessarily reflect a change in kinetics. The fact that all the oral elimination profiles were represented as a single exponential while some of the intraruminal elimination profiles required the sum of two exponentials may be due to operational limitations. With more frequent blood sampling near the peak levels, it is possible that an extra exponential could be estimated for all oral and intraruminal profiles. The apparent difference in elimination rates may also be an artifact of the blood sampling schedule. Terminal sampling times for oral and intraruminal routes were 96 and 168 h respectively. If the actual specific activities for the oral route at 168 h were higher than the extrapolated values based on observed elimination rates up to 96 h, elimination rates would be more similar.

The reduced availability and the slower elimination of tocopherol administered via the oral, intraruminal and intramuscular routes compared with the intravenous route are composites of many factors including amount absorbed, quantity of lipoprotein available for tocopherol transport and recycling of the radioisotope from other compartments into the plasma. According to Gallo-Torres & Miller (1971), the rate of transfer of vitamin E from the plasma is markedly influenced by its solubility and partition coefficients between aqueous and lipid phases. Hence the vehicle (ethanol) used in the oral, intraruminal and intramuscular routes could affect α-tocopherol passage through cellular membranes. According to Bateman & Vccellini (1984, 1985) the vehicle in which vitamin E is dissolved dramatically affects the vitamin's uptake into the bloodstream. These workers observed a rapid uptake of vitamin E into the bloodstream of man after oral administration of a water-soluble form. To contribute to the understanding of nutrition and to determine its absorption more accurately, work is needed on the use of various vehicles in which the vitamin E is administered into the ruminant animal.

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