

Monitoring vitamin E pools in sheep tissue and plasma after intravenous dosing of radiotocopherol

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The fate of radiotocopherol was studied in plasma and tissues of sheep at various intervals after injection of single intravenous doses of ^3H -labelled D- α -tocopherol. Plasma samples were taken at regular intervals after dosing and selected tissues were taken from all sheep after slaughter and assayed for radioactivity and D- α -tocopherol. Sheep were killed in groups of five at 24, 72, 96, 272 and 432 h post-dosing. Plasma profiles were characterized as a sum of three exponential terms. A principal component analysis of tissue concentrations was carried out to identify tissues with parallel profiles of log (disintegrations/min per μg) over time. Five groups of tissues with distinct uptake and elimination processes were identified. The D- α -tocopherol in the liver and heart appeared to be consistent with the post-distributive kinetics of a highly perfused shallow compartment, while lung kinetics appeared to reflect a non-linear kinetic process. The third group, which included the spleen, neck brachiocephalic muscle and pancreas, had depletion rates parallel to those of plasma for 24–272 h, but slower decreases than plasma over 272–432 h. Hip gluteus muscle and kidney comprised a fourth group, with depletion parallel to plasma rates for 24–96 h but progressively slower than plasma decreases over 96–272 h. Adrenal kinetics resembled the fourth group, but had a more rapid decrease in specific activities over 24–72 h.

Vitamin E: Sheep

According to Sternberg & Pascoe-Dawson (1959), the use of labelled α -tocopherol offers an immediate means of estimating the turnover of α -tocopherol in the tissues of rats. Previous work (Hidiroglou & Karpinski, 1987) showed that some insight into the fate of tocopherol in the body of sheep may be gained by the use of radiotocopherol. This permitted the evaluation of the rate of appearance or disappearance, as well as the pool size for sheep plasma. The object of the present paper is to provide a more complete description of the fate of intravenously injected radiotocopherol in sheep by measuring its rate of accumulation and elimination from tissues, thereby characterizing the intensity of its metabolism (Gallo-Torres & Miller, 1971).

EXPERIMENTAL

Animals

Twenty-five yearling crossbred (Finnish Landrace \times Shropshire \times Suffok) wethers, weighing 45–50 kg, were used. All animals originated from one flock and were born and raised in confinement. For 6 months before and during the experiment, the animals were fed on a

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diet *ad lib.* consisting of (g/kg): grass silage 400, hay 400 and maize silage 200. At 10 d before dosing, the sheep were placed in metabolism cages. Five animals were randomly assigned to each of five groups. Sheep in groups 1, 2, 3, 4 and 5 were killed at 24, 72, 96, 272 and 432 h respectively after a single intravenous dose of 4 μ Ci/kg body-weight of 3 H-labelled D- α -tocopherol dissolved in emulsion (Hidiroglou & Karpinski, 1988). Injections were given directly into the right jugular vein. Blood samples (10 ml) were taken from the left jugular vein at preset intervals (see Fig. 1, p. 378) 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. After completion of the sampling period, the wethers were killed by intravenous injections of sodium pentobarbital. Selected tissues (heart, hip gluteus and neck brachiocephalicus muscle, lung, liver, spleen, pancreas, kidney, adrenals) were taken from all sheep after slaughter. All the tissues were rinsed immediately with water and dried with filter paper. Care was taken to dissect away attached adipose tissue. Analyses of livers were performed immediately after removal from wethers. The other tissues were stored at -20° until required.

Materials

Standard D- α -, D- β -, D- γ - and D- δ -tocopherols were 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. The purity of the tocopherols was not less than 95%, as determined by thin-layer chromatography on silica gel in chloroform.

Analytical methods

Tocopherol determination in roughage was performed by high-performance liquid chromatography (McMurray & Blanchflower, 1979). Identification and quantification of the D- α -, D- β -, D- γ - and D- δ -tocopherols were by comparison of retention times and peak areas with tocopherol standards. Quantification of vitamin E in sheep plasma and tissues was performed by HPLC equipped with a Perkin-Elmer 650-150 fluorescence outfit with a microflow-cell unit. Tissue samples for vitamin E measurements were prepared according to Burton *et al.* (1985).

Analysis of radioactivity

Triplicate samples (0.5 ml portions) of plasma were assayed for total radioactivity in 10 ml of a phase-combined system (Beckman 3801 with Aquasol 2 scintillation fluid; New England Nuclear, Boston, MA). Fresh samples (50-100 mg) of tissue were placed on ashen cellulose pellets (450 mg) and burned on a Packard Model 306 sample oxidizer (Packard Instrument Co., Downes Grove, IL). The tritiated water (primed) was dissolved in Monophase-40 (scintillation fluid; Packard), added automatically by the sample oxidizer. The recovery rate of 3 H by this procedure was found to be 99%. All samples were counted in a Beckman LS 280 liquid-scintillation spectrometer (Beckman Instrument Ltd, Fullerton, CA), 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).

Statistical methods

Characterization of the plasma D- α -[3 H]tocopherol specific activity profiles was based on non-linear least squares applied to three-compartment pharmacokinetic models. For estimation purposes the models were represented as sums of exponentials in the form:

$$\log c(t) = \log \left[\sum_{i=1}^3 A_i e^{-k_i t} \right] + e(t),$$

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

(Estimates with their standard errors)

Group 5 sheep no.		$\log(c(t)) = \log [A_1e^{-k_1t} + A_2e^{-k_2t} + A_3e^{-k_3t}]$						
		Intercept (dpm/ μg)			Exponent (/h)			D- α -tocopherol ($\mu\text{g}/\text{ml}$)
		A_1	A_2	A_3	k_1	k_2	k_3	
1	Mean	93433	29440	2781	2.2157	0.0640	0.0051	1.27
	SE	8622	1843	165	0.4373	0.0043	0.0002	0.08
2	Mean	91463	31024	3599	1.3245	0.0558	0.0051	1.46
	SE	8775	2609	260	0.2865	0.0047	0.0003	0.06
3	Mean	103284	33515	4612	1.6468	0.0950	0.0058	1.37
	SE	9697	3158	256	0.3453	0.0107	0.0002	0.11
4	Mean	115154	22835	4413	0.9965	0.0358	0.0074	1.09
	SE	7175	1685	378	0.1198	0.0028	0.0003	0.10
5	Mean	122401	22697	4464	1.6268	0.0579	0.0065	1.20
	SE	6743	1303	202	0.1716	0.0041	0.0002	0.09

t , time; dpm, disintegrations/min.

where $c(t)$ denotes the specific activity of D- α -[^3H]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. The logarithmic transformation was required in order to stabilize variances since, according to preliminary analyses, the standard deviations were approximately proportional to the observed concentrations. Parameter estimation was carried out using the SAS non-linear regression procedure (SAS Institute, 1985) with the Marquardt iteration method.

Estimates were also derived for the following parameters (Gibaldi & Perrier, 1975): the half-life ($t_{1/2}$) of the terminal elimination phase, $t_{1/2} = 0.693/k_3$; the volume of the central compartment (V_c),

$$V_c = \frac{\text{dose}}{\sum_i A_i} / \alpha\text{-tocopherol level};$$

the clearance rate (CR),

$$\text{CR} = \frac{\text{dose}}{\sum_i (A_i/k_i)} / \alpha\text{-tocopherol level};$$

and the transport rate (TR),

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

For these calculations dose was expressed in dpm (i.e. $1 \mu\text{Ci} = 2.2 \times 10^6$ dpm). Specific activity and α -tocopherol levels were measured in dpm/ μg and $\mu\text{g}/\text{ml}$ respectively. Thus, V_c , TR and CR were expressed in terms of litres, $\mu\text{g}/\text{h}$ and ml/h respectively.

As with the plasma, specific activities of D- α -[5-Me- ^3H]tocopherol in tissues were expressed in terms of dpm/ μg D- α -tocopherol and were analysed on the logarithmic scale. Analyses were carried out to identify tissues which had parallel profiles of $\log(\text{dpm}/\mu\text{g})$ over time. Each set of tissue and plasma measures within a sheep was regarded as a multi-variate observation. Tests of parallelism were based on a multi-variate profile analysis

Table 2. Kinetic parameter estimates* of $D\text{-}\alpha\text{-}[^3\text{H}]\text{tocopherol}$ specific activity in sheep plasma after intravenous injection of $D\text{-}\alpha\text{-}[^3\text{H}]\text{tocopherol}$

Group 5 sheep no.	Central compartment volume (l)	Terminal elimination phase half-life (/h)	Transport rate ($\mu\text{g}/\text{h}$)	Clearance rate (ml/h)
1	2.6	136	420	306
2	3.2	136	331	304
3	2.1	120	363	249
4	2.4	94	326	258
5	2.4	107	381	317
Average	2.6	118	364	287
SD	0.40	18	39	31

* Estimates are based on the three-exponential equation estimates given in Table 1.

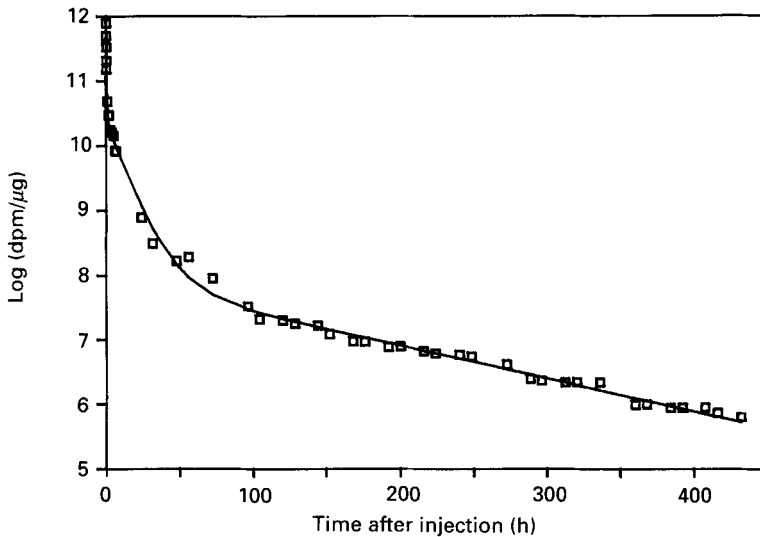


Fig. 1. Profile of observed and predicted log plasma concentrations of $D\text{-}\alpha\text{-}[^3\text{H}]\text{tocopherol}$ after intravenous injection: group 5, sheep no. 1. (For details of procedures, see p. 376.)

(Timm, 1975). A principal component analysis (Snee *et al.* 1979) identified two linear combinations (principal components) of the variables which contained most of the information concerning non-parallelism. A plot of the two principal components (Timm, 1975) was used to identify groups of tissues with parallel $\log(\text{dpm}/\mu\text{g})$ profiles. Tissues within a group will have specific activities which are approximately proportional at all times, implying that these tissues have similar rate constants of uptake and removal from the system and transfer to circulation (Gurpide & Mann, 1970).

Table 3. Mean D- α -tocopherol concentrations in tissues ($\mu\text{g/g}$ fresh tissue) and plasma ($\mu\text{g/ml}$) of sheep at different times of killing after an intravenous injection of D- α -[^3H]tocopherol

	Time of killing (h)					Coefficient of variation (%)*
	24	72	96	272	432	
Heart	6.1	6.2	6.2	5.9	6.3	19
Hip muscle	3.0	3.4	3.1	2.7	3.3	14
Neck muscle	2.4	3.2	2.8	3.4	3.5	15
Lung	8.2	11.3	7.9	7.1	6.6	17
Liver	7.1	6.9	7.2	6.0	6.6	11
Spleen	4.3	5.6	5.5	5.0	5.0	19
Pancreas	15.1	16.1	15.4	16.7	17.4	12
Kidney	5.7	4.7	4.6	4.1	4.5	20
Adrenal	33.9	32.2	†	33.0	30.4	8
Plasma	1.4	1.1	1.1	1.0	1.2	20

* Based on the pooled standard deviation and the overall mean.

† No samples were available at 96 h.

RESULTS

The plasma D- α -[^3H]tocopherol profiles in group 5 sheep (killed at 432 h) were represented as a sum of exponentials and attempts were made to fit higher-order models sequentially. The adequacy of each fitted model was verified through examination of least squares residuals and goodness-of-fit F tests. Profiles were best represented as a sum of three exponentials:

$$c(t) = A_1e^{-k_1t} + A_2e^{-k_2t} + A_3e^{-k_3t} \quad (1)$$

and fitted in the form

$$\log(c(t)) = \log[A_1e^{-k_1t} + A_2e^{-k_2t} + A_3e^{-k_3t}]$$

Parameter estimates for each animal are presented in Table 1 along with average D- α -tocopherol levels in the plasma over the blood sampling period. Estimates of $t_{1/2}$, V_c , TR and CR are provided in Table 2. Typical observed and predicted profiles (group 5, sheep no. 1) are displayed in Fig. 1.

The first phase of the profile (i.e. $A_1e^{-k_1t}$) appeared to be a distributive phase which was clearly evident for 25–28 min. Beyond this point the decay profile was dominated by the second and the third exponential terms (i.e. $A_2e^{-k_2t} + A_3e^{-k_3t} > A_1e^{-k_1t}$). The second phase (i.e. $A_2e^{-k_2t}$) was evident for up to 50 h in some sheep (i.e. $A_2e^{-k_2t} > A_3e^{-k_3t}$). After 165 h all profiles were essentially in the terminal decay phase represented by $A_3e^{-k_3t}$.

Profiles for sheep in the other four groups appeared to be consistent with eqn (1). However, satisfactory parameter estimates generally could not be derived since the earlier times of killing resulted in truncation of profiles so that the post-distributive phases were not adequately represented.

Tissue D- α -tocopherol levels (Table 3) indicated that the pancreas and particularly the adrenal gland had the largest D- α -tocopherol concentrations with 16.1 and 32.4 $\mu\text{g/g}$ respectively. Mean levels in the other tissues ranged from 3.0 to 8.1 $\mu\text{g/g}$. Tissue levels were reasonably stable across the five groups.

Tissue mean specific activities are given in Table 4. A test of constant proportionality

Table 4. Mean specific activities (dpm/ μg D- α -tocopherol means) in tissues of sheep at different times of killing after an intravenous injection of D- α -[^3H]tocopherol

	Time of killing (h)					Coefficient of variation (%)*
	24	72	96	272	432	
Heart	761	381	425	216	111	25
Hip muscle	425	294	258	148	107	20
Neck muscle	600	465	529	143	117	20
Lung	2193	672	1042	777	403	28
Liver	2515	1220	1768	717	305	22
Spleen	1078	621	580	246	164	18
Pancreas	216	135	119	62	38	27
Kidney	672	444	468	237	221	27
Adrenal	729	299	†	143	151	14

* Based on the pooled standard deviation and the overall mean.

† No samples were available at 96 h.

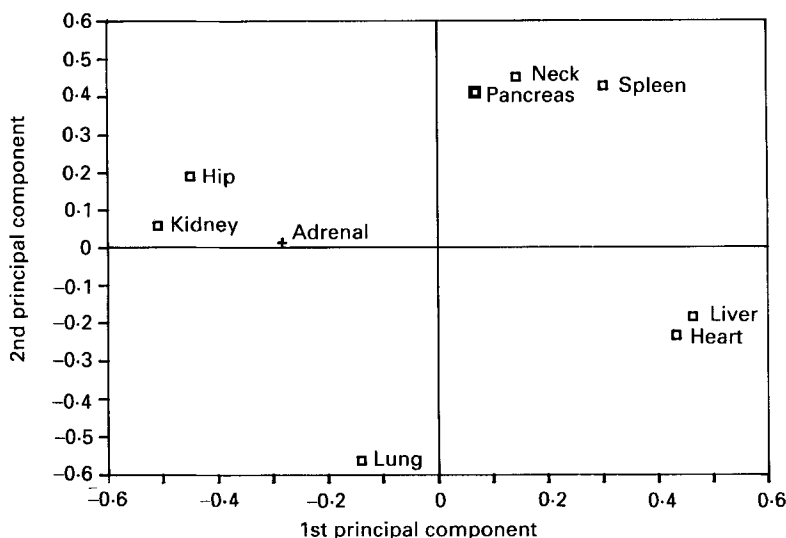


Fig. 2. Plot of the first two principal components identified in the principal component analysis.

among tissues at all times is equivalent to a test of parallelism of tissue $\log(\text{dpm}/\mu\text{g})$ profiles. The parallelism test was highly significant ($P < 0.0001$, multi-variate profile analysis) indicating that the tissues cannot be lumped into one group with regard to D- α -tocopherol kinetics. The plasma D- α -[^3H]tocopherol profiles also indicated that at least two peripheral groups were required to characterize the plasma kinetics. A measure of the lack of parallelism in the tissues is provided by the deviations:

$$r_{gt} = y_{gt} - y_g - y_t + y \quad g = 1, \dots, 5; t = 1, \dots, 9,$$

where y_{gt} denotes the $\log(\text{dpm}/\mu\text{g})$ in group g for tissue t and y_g , y_t and y denote group, tissue and overall averages of y_{gt} respectively. A summary measure of non-parallelism is provided by the usual analysis of variance sum of squares for group \times tissue interaction.

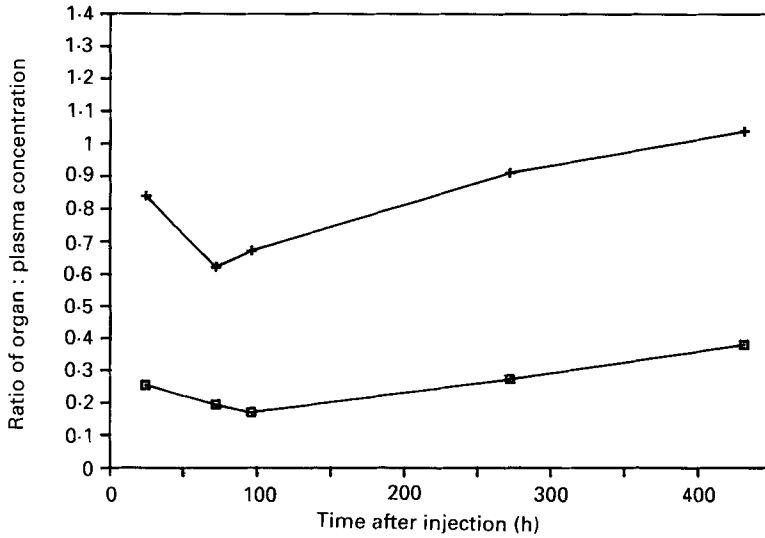


Fig. 3. Profiles of ratio of organ : plasma $D\text{-}\alpha\text{-}[^3\text{H}]\text{tocopherol}$ concentrations for the liver (+) and heart (□) after intravenous injection.

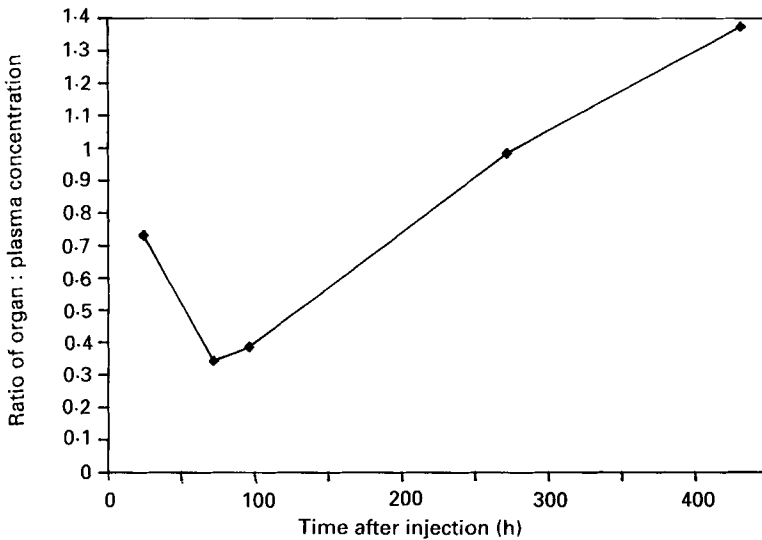


Fig. 4. Profiles of ratio of organ : plasma $D\text{-}\alpha\text{-}[^3\text{H}]\text{tocopherol}$ concentrations for the lung (◆) after intravenous injection.

This interaction sum of squares can be partitioned by a principal component analysis. A principal component analysis of the 5×9 matrix of deviations r_{gt} identified two linear combinations (the first two principal components) of the tissues which explained 80% of the variability in the r_{gt} . These two principal components are plotted in Fig. 2. Tissues with parallel profiles will have similar coefficients in the principal components and appear in clusters in Fig. 2. Note that the principal component analysis initially did not include the

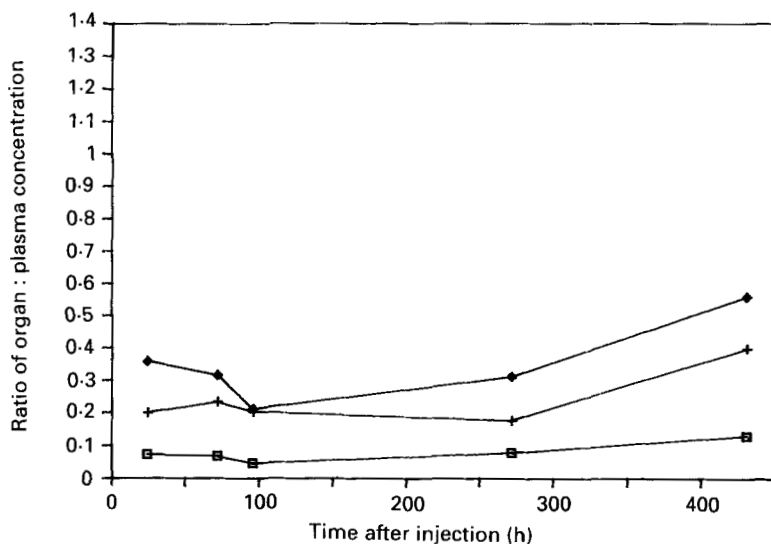


Fig. 5. Profiles of ratio of organ or tissue: plasma D- α -[^3H]tocopherol concentrations for spleen (◆), neck muscle (+) and pancreas (□) after intravenous injection.

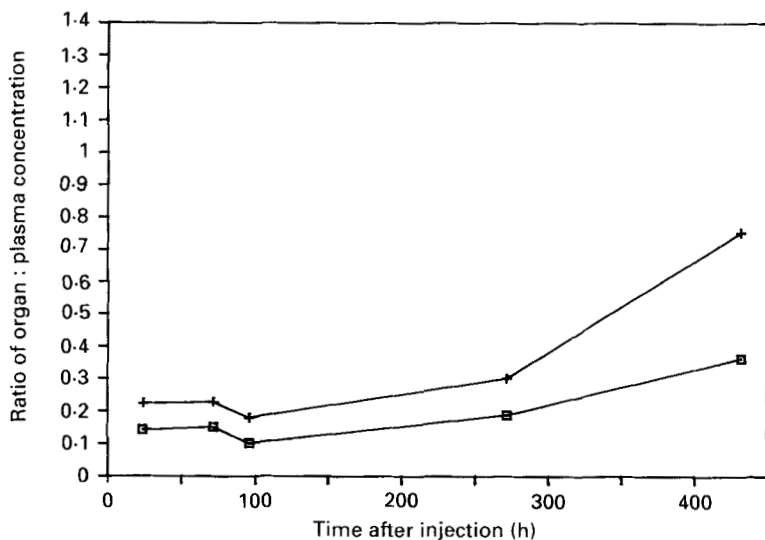


Fig. 6. Profiles of ratio of organ or tissue: plasma D- α -[^3H]tocopherol concentrations for kidney (+) and hip muscle (□) after intravenous injection.

adrenal tissue since a complete set of measures was not available (96 h measure was missing). A second principal component analysis based on the 24, 72, 272 and 432 h measurements was carried out in order to evaluate the adrenal tissue with the corresponding observations for other tissues. These results were consistent with the first principal component analysis and the value for adrenal tissue from the second analysis is also given in Fig. 2.

Five groups or mathematical compartments are suggested by Fig. 2: (1) liver, heart; (2)

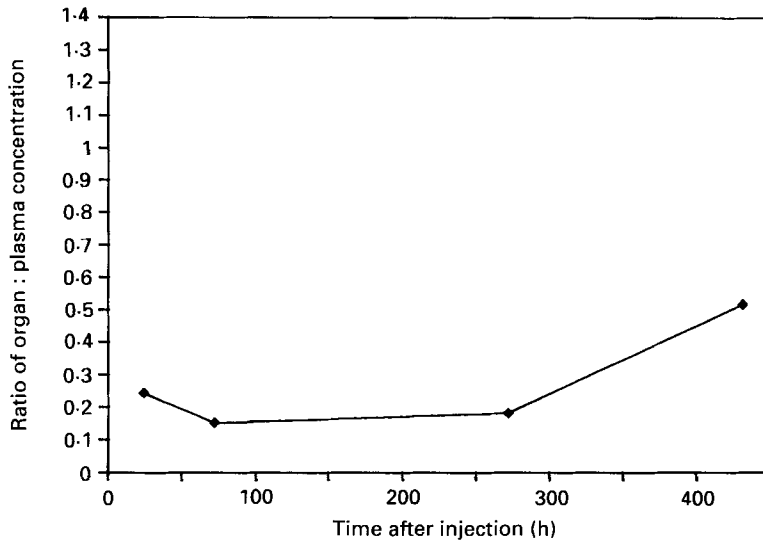


Fig. 7. Profiles of ratio of organ : plasma $D\text{-}\alpha\text{-}[^3\text{H}]\text{tocopherol}$ concentrations for adrenals (\blacklozenge) after intravenous injection.

pancreas, neck, spleen; (3) lung; (4) hip, kidney; (5) adrenal. Multi-variate profile analyses were carried out to verify the suggested groupings. In each case tissues within a group had parallel profiles ($P > 0.25$). Broader groupings were also considered but the profile analyses indicated that further grouping of tissues was inappropriate (non-parallelism $P < 0.06$). All tissues showed a general decline (Table 4) in specific activities over the 24–432 h observation period, but the heart, neck, lung, liver and kidney all showed an increase in activity at 96 h compared with 72 h. Rates of depletion varied among the identified compartments and an indication of the comparative depletion rates is available from plots of specific activities in tissues relative to the corresponding plasma levels *v.* the time of killing (Figs 3–7).

DISCUSSION

Pharmacokinetic studies have frequently been based on analyses of plasma profiles since plasma samples are relatively easy to obtain at frequent intervals following administration of a chemical. Generally a multiphasic decline of plasma concentrations is observed which can be represented as a sum of exponential terms. Characterization of $D\text{-}\alpha\text{-}[^3\text{H}]\text{tocopherol}$ plasma profiles in the present study as a sum of three exponentials was consistent with previously reported results (Hidioglou & Karpinski, 1987). Differences in observed elimination in the two studies are at least partly due to the differences in the plasma sampling periods. The extended plasma sampling in the current study (432 h *v.* 96 h) naturally provides a more reliable estimate of terminal phase elimination which appeared to be considerably slower than that previously reported (k_3 0.0060 h *v.* 0.0138 h; Hidioglou & Karpinski, 1987). Elimination over the extended period 96–432 h may actually represent a slower elimination phase which should theoretically be represented by a fourth exponential term in the plasma equation. However, attempts to fit a higher-order exponential equation were not successful since the iterative non-linear least squares estimation process would not converge. Consequently, the inter-related parameter estimates of the A_i and k_i in the exponential equation all had reduced values in the present study. This

is a characteristic of multiphasic profiles which are represented by the same equation when different sampling periods and reduced terminal elimination rates are observed.

Following the estimation of an exponential equation, the conventional treatment of plasma values invokes a simple compartmental model, such as a mamillary (open, fully interchanging multi-pool) model with elimination assumed to occur exclusively from the central (plasma) compartment. This approach had been previously applied to plasma D- α -[^3H]tocopherol values (Hidiroglou & Karpinski, 1987) to derive some insight into the kinetics of vitamin E. The shallow peripheral compartment was portrayed as a composite of highly perfused tissues and organs, while the less perfused regions were mathematically represented by the deep peripheral compartment. However, it is generally realized (Gurpide & Mann, 1970) that a wide variety of pharmacokinetic models with three or more compartments would be equally compatible with the observed plasma values and that the mathematical simplification is simply a convenient tool for deriving additional information from an otherwise intractable problem.

The present study allowed a more physiologically relevant approach (Mintun *et al.* 1980) which was based on direct observation of concentrations in the various tissues. Tissues with proportional specific activities over the 24–432 h observation period were considered to have similar kinetic behaviour. Five groups of tissues were identified as constituting peripheral compartments with distinct uptake and depletion processes. Kinetics in the liver and heart were fairly close to plasma kinetics, as indicated by the relatively horizontal straight lines in Fig. 3 and profile analysis tests for time differences. The profiles appeared to be consistent with the post-distributive kinetics of a highly perfused shallow compartment in a mamillary system. Liver specific activities were close to plasma levels (ratio approximately 1.0) (Fig. 3) over the entire sampling period and exceeded plasma levels at 432 h. Bieri (1972) observed two phases in the decay curves of vitamin E in rat tissues. The first phase suggested the existence of a pool of labile α -tocopherol which is rapidly metabolized. This was most pronounced in the liver and heart tissues which, according to Bieri (1972), were in equilibrium with the plasma in rats. The kinetic studies on tissue uptake and depletion carried out by Machlin & Gabriel (1982) suggested that the liver is the major storage organ for tocopherol and helps to maintain vitamin E levels when the intake of vitamin E becomes inadequate. According to Murphy & Mavis (1981) there is evidence for a hepatic cytosolic α -tocopherol-binding protein.

In the present experiment no attempt was made to identify the possible metabolites which retained the ^3H of the 5-methyl group following administration of the DL- α -[^3H]tocopherol. In a previous experiment (Hidiroglou *et al.* 1970) we reported that following oral administrations of the DL- α -[^3H]tocopherol to sheep, 95% of the liver radioactivity was identified as α -tocopherol. Krishnamurthy & Bieri (1963), and Plack & Bieri (1964), reported that oral and intraperitoneal administrations of α -[^{14}C]tocopherol to rats and chicks gave rise mostly to unchanged tocopherol in the tissue of the fetal animals.

The kinetics of uptake and elimination of D- α -tocopherol from the lung (Fig. 4) are quite distinct in that specific activities decreased more rapidly than in plasma for 24–72 h and much more slowly for 72–432 h. By 272 h lung specific activities exceeded plasma levels. This behaviour is not consistent with a mamillary model and suggests a non-linear kinetic process. Knight & Roberts (1986) reported that following intravenous administration of α -[^{14}C]tocopherol to newborn rabbits, the lung followed the liver in the highest percentage (12 (\pm SE 6)%) of radioactive material. This distribution of the injected labelled vitamin E was not surprising since the lung has an abundant blood supply.

For the spleen, neck muscle and pancreas (Fig. 5) depletion rates were parallel to those for plasma for 24–272 h. However, this group had slower decreases than plasma over 272–432 h. Hip muscle and kidney (Fig. 6) depletion paralleled plasma rates for 24–96 h,

but became progressively slower than plasma decreases over 96–272 h. A continued increase in tissue levels relative to plasma levels during the post-distributive phase is characteristic of deeper peripheral compartments. However, the kinetics were not consistent with a mamillary linear system since levels appeared to be in equilibrium with plasma for a prolonged period before the change in depletion rates at 96 h and 272 h. Adrenal kinetics (Fig. 7) resembled hip muscle and kidney kinetics but had a more rapid decrease in specific activities over 24–72 h.

A useful alternative to the above approach (e.g. Wastney *et al.* 1986) is to derive estimates of intercompartment transfer and elimination rates for all tissues simultaneously by applying a sequential simulation–estimation procedure (Berman *et al.* 1983) to a linear compartment configuration. However, the procedure requires good initial estimates of the various intrinsic volumes, blood flow-rates, partition coefficients and biochemical constants specific to the chemical under investigation. Since modelling in the present study was based on tissue samples obtained from different animals at different time-points, the problem was further complicated by inter-animal and temporal variation. Since insufficient information was available in our study to model tissue profiles, depletion rates were simply compared with the plasma rates.

The heart, liver and lung appeared to have rapid uptake and turnover of D- α -tocopherol. Together with plasma these tissues accounted for 69% of the radioactivity at 24 h and 58% at 432 h. However, these tissues had only moderate D- α -tocopherol concentrations (6.8–8.2 $\mu\text{g/g}$). The pancreas and adrenal accounted for a small percentage of radioactivity (< 10) but had the largest D- α -tocopherol concentrations (16.2 and 25.9 $\mu\text{g/g}$ respectively). The other organs appeared to have a relatively minor impact on body D- α -tocopherol levels since both their concentrations and radioactivity levels were comparatively low. As reported for rat tissues (Aftergood *et al.* 1976), the capacity of sheep muscle tissue to store vitamin E appeared to be limited. The considerable variation in the uptake and depletion of the different tissues may be related to the rate of permeation of α -tocopherol into the cellular compartments of the various tissues and organs.

The present work is contribution no. 1575 of the Animal Research Centre, Canada.

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