A study of whole-body isotope dilution of [^{14}C]ascorbic acid in guinea-pigs with graded ascorbate intakes

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The purpose of the present study was first to assess the extent to which unlabelled ascorbate in the diet of guinea-pigs can exchange with labelled ascorbate within their organs when the dietary intake is varied over a wide range, and second to determine whether the retention of label might be used to assess either the amount of ascorbate intake or its biological availability where these are not known. The retention of [^{14}C]ascorbate in the body and in various organs of guinea-pigs were, therefore, measured following a 13 d period of graded dietary intakes of ascorbate. It was found first, that the amount of label retained in each of the organs, 13 d after the initial dose of labelled ascorbate, was much more closely related to the amount of ascorbate intake after labelling than to the intake (and tissue ascorbate levels) before and at the time of labelling. Second, most of the individual internal organs exhibited a constant relationship between the specific activity at 13 d and the dietary intake, except for brain which was flushed to a smaller extent. Third, in agreement with several previous studies a high proportion of the radioactive label in the tissues was found to be still present in ascorbate. The specific activity of column-purified ascorbate was very similar to the estimated specific activity in the crude extract, which implies that it may be possible to estimate specific activities (or stable isotope enrichments) at certain sites without rigorous isolation procedures. Fourth, the amount of radioactivity appearing in the urine 2 d before killing the animals was correlated with the amount of ascorbate intake and with tissue specific activities, suggesting that intakes (or bioavailability) might be predicted from the patterns of label-appearance in the urine.

Vitamin C: Isotope labelling: Guinea-pig

The use of 1-[^{14}C]ascorbic acid as a probe of the metabolism, in vivo, of ascorbic acid extends back to the 1950s, and has proved a very useful tool to answer questions about ascorbate economy especially in those species, such as guinea-pigs and higher primates, which cannot synthesize ascorbate from other sugars.

A number of these studies have focused on the use of this label to measure body pool size and turnover rates (Burns *et al.* 1951; Salomon, 1957, 1962; Abt & von Schuching, 1961; Ginter *et al.* 1971; Hornig *et al.* 1973; Hornig & Weiser, 1976; Tillotson, 1980; Hornig & Hartmann, 1982; Kipp & Rivers, 1984; Zloch & Ginter, 1988), and parallel studies have also been performed on human subjects (Atkins *et al.* 1964; Baker *et al.* 1969; Kallner *et al.* 1979).

Less attention has, however, been paid to the question of the relationship between usual dietary intake of unlabelled ascorbate and the amount from a single dose of label that is retained by the tissues or is excreted in the urine. This question may have an important practical relevance in several respects, including the search for new methods of assessing intakes and the biological availability of ascorbate from diets. There remains some uncertainty about the feasibility of predicting ascorbate intakes from ascorbate-label

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retention by guinea-pigs: the studies of Tillotson (1980), in particular, have highlighted the problem of variable oxidation rates and their dependence on stress, which apparently can swamp the influence of variations in dietary intake.

The main purpose of the present study was to test the feasibility of using the retention of a single oral dose of ascorbate-label in various guinea-pig tissues and its excretion as urinary degradation products, as possible indices of dietary intake of unlabelled ascorbate. The tissue retention and distribution of label were measured 13 d after an oral labelling dose, this time-interval being chosen to ensure that the early inter-tissue redistribution effects would be minimized, and that the cumulative effect of the dietary intake over a substantial period of time would be maximized. In addition, the effects of variations in ascorbate body pool size before the labelled dose were compared with the effects of variations in ascorbate intake subsequent to the labelled dose.

MATERIALS AND METHODS

Two studies were performed (Table 1). The first employed a relatively low dose of labelled ascorbate with two or three animals in each dietary group; this protocol established the characteristic patterns of response for the individual tissues. The specific activities here were, however, too low for it to be feasible to perform column separations and, thus, to check the identity of the labelled products. The second study employed only two animals per dietary group and fewer groups, but it used larger amounts of label in each animal, thus permitting the picture to be extended by a column chromatography identity-check.

Animals and diets

Male Dunkin–Hartley guinea-pigs weighing 272 (SE 13) g were acclimatized to synthetic powdered diets containing varying amounts of ascorbate for 2 weeks before being dosed with the labelled ascorbate. They were caged singly in suspended wire cages with a 12 h light–dark cycle and were given food and water *ad lib.*

The basic diet was that used in previous studies (Bates & Cowen, 1988; Bates *et al.* 1988), with 0·043 g iron (as ferrous citrate)/kg but without additional hay. Ascorbic acid was added in amounts from 0·05 to 5·0 g/kg diet; the diets were stored at 4° and fresh batches were prepared every 2 weeks. Portions of the diets were analysed for their vitamin C content by the method of Vuilleumier & Keck (1989), and the mean concentrations are shown in Table 1.

During the 14 d acclimatization period before the dose of labelled ascorbate, the animals were subdivided into five initially weight-matched groups to receive diets containing ascorbate (g/kg): 0·1 (A), 0·25 (B), 0·5 (C), 1·0 (D) and 5·0 (E) (nominal levels). The labelled ascorbate (see below) was given by syringe into the mouth in a sucrose solution (10 g/l), and the animals were then sub-divided again to receive graded amounts of dietary ascorbate (see Table 1). At 3 or 4 d intervals after dosing with label, urine samples were collected in metabolism cages over a 6 h daytime interval into 0·5 g oxalic acid crystals to prevent degradation of any ascorbate present. After 13 d the animals were killed by diethyl ether anaesthesia; blood was then obtained by cardiac puncture in heparinized syringes, and the internal organs were removed for vitamin C extraction. Intestine was slit and rinsed free of its contents before extraction.

[^{14}C]*ascorbate dose*

1-[^{14}C]ascorbic acid was obtained from New England Nuclear (Du Pont (UK) Ltd, Stevenage, Herts). The material used in Expt 1 had a specific activity of 4·90 Ci/mol, and that used in Expt 2 had a specific activity of 2·47 Ci/mol. The purity was checked by

Table 1. *Expts 1 and 2. Group allocations and analysed dietary contents of ascorbate at each stage*

Expt 1				Expt 2			
Group designation	No. per group	Dietary ascorbate before label administration (predose diet) g/kg	Dietary ascorbate after label administration (post-dose diet) g/kg	Group designation	No. per group	Dietary ascorbate before label administration (predose diet) g/kg	Dietary ascorbate after label administration (post-dose diet) g/kg
Group 1							
1A1	2	0·098	0·025	2A1	2	0·104	0·040
1B1	2	0·19	0·025				
1C1	2	0·56	0·025				
1D1	2	1·17	0·025	2D1	2	0·91	0·040
Group 2							
1A2	3	0·098	0·095	2A2	2	0·104	0·073
1B2	3	0·19	0·19	2B2	2	0·28	0·23
1C2	3	0·56	0·42	2C2	2	0·49	0·41
1D2	3	1·17	1·05	2D2	2	0·91	0·78
1E2	3	5·54	5·25	2E2	2	5·65	4·50

Each animal in each experiment received the predose diet for 2 weeks. It was then given the labelled ascorbate as a single oral dose and was immediately transferred to the new (post-dose) diet, which for group 1 was at a constant low level of ascorbate, whereas for group 2 it remained essentially similar to the levels that each subgroup (A–E) had already received during the predose period. In Expt 1, the dose to each animal was 2 μCi (0·4 μmol); in Expt 2 it was 17 μCi (7·0 μmol), slightly adjusted between animals to ensure a constant dose per unit body-weight.

descending paper chromatography, the eluent being *n*-butanol–glacial acetic acid–water 4:1:5, by vol.). Essentially all the radioactivity was present in a single peak, which also decolorized 2,6-dichlorophenol indophenol dye, as predicted.

Extraction and analysis of [^{14}C]ascorbate from guinea-pig tissues

After removal, each organ was immediately homogenized in a fresh aqueous solution of metaphosphoric acid (50 g/l) using between 3 and 10 vol. per unit fresh weight of tissue. For plasma, 1 vol. metaphosphoric acid (100 g/l) was used. After removal of the insoluble material by centrifugation for 20 min at 3000 ***g***, the supernatant extracts were stored frozen at −25° for 2–3 weeks.

Total radioactivity in the extracts and urine samples was measured in LKB Optiphase Hisafe 3 scintillation fluid, with a Packard Minaxi Tricarb 4000 scintillation counter. Total ascorbic acid (including dehydroascorbate) was measured on a Cobas Bio centrifugal analyser, by the method of Vuilleumier & Keck (1989).

In Expt 2, some of the extracts were further analysed by reverse-phase ion-pair high-pressure liquid chromatography (HPLC), to ascertain the identity of the radioactive material. For this analysis, the main column was a 250 × 8 mm Techoprep 5–20 μm C_8 (HPLC Technology, Macclesfield, Cheshire) with a precolumn, 50 × 3 mm, C_{18}-Corasil. The eluent (isocratic; adjusted to pH 4·0) contained (mmol/l): sodium chloride 10, Na_2EDTA 0·6, sodium acetate 140, *n*-hexadecyltrimethylammonium bromide 10, methanol 100 ml/l, as described previously (Bates *et al.* 1988). The flow-rate was 4·0 ml/min, and

ascorbate was detected by a BAS (Bioanalytical Systems, Lafayette, Indiana, USA) electrochemical detector set at 0·6 V, with 50 nA or 500 nA sensitivity. Samples containing metaphosphoric acid were mixed with an equal volume of double-strength eluent and were centrifuged to remove precipitated material before injection. The injection loop volume was 1·0 ml. Eluted material was collected, usually in five fractions between 5 and 10 ml in volume, the fourth of which was arranged to coincide with the detector peak of ascorbic acid; this was well-separated from the column void volume. The fractions were assayed for radioactivity as described previously, and the recovery of radioactivity in the ascorbate peak was compared with the amount injected and with the amount appearing in the other four fractions. It was demonstrated in preliminary runs that ascorbic acid was well-separated from dehydroascorbate (eluting earlier) and from oxalate (eluting later); thus, the main degradation products that were likely to retain the ^{14}C label were readily distinguished from the ascorbate peak.

RESULTS

Body-weight

Fig. 1 shows the increases in body-weight for each sub-group in each experiment. Clearly all sub-groups gained weight at about the same rate during the experiment; the slightly poorer performance of groups 1A1–1D1 than those of groups 1A2–1E2 in Expt 1 was not seen in groups 2A1 and 2D1 of Expt 2. Both experiments exhibited a small hiatus in weight gain following the ^{14}C dose.

Concentrations of ascorbic acid in the tissues at the end of each experiment

Fig. 2 shows the relationship between dietary ascorbate level and mean tissue concentrations of ascorbate for Expt 1; Fig. 3 shows the same relationship for Expt 2. Because of the wide range of values, especially between different tissues, and the wide range of dietary ascorbates, both axes have been converted to a log scale. The conclusions to be drawn from these comparisons, which showed very similar patterns for each of the two experiments, are: (1) apart from the brain, all the tissue sites sampled showed a steep rise in the ascorbate level with increasing dietary ascorbate at the lower end of the intake range, but they all approached a constant level between 1 and 5 g/kg in the diet. The brain, as expected, showed a less steep rise at the lower end of the intake range, but showed the same approach to a plateau at the higher intakes; (2) group 1 animals, which had been switched over to the lowest vitamin C intake 13 d before being killed, exhibited a small residual effect of the dietary vitamin C levels during the preceding 2 weeks. Thus, a 13 d period seems not to be sufficient to attain a new tissue equilibrium with intake, although the mean ascorbate concentration ratio for group A: group D tissues was only 1·79 in group 1, compared with 5·63 in group 2, indicating partial equilibration between the new intakes and the tissue concentrations in group 2.

Distribution of ascorbic acid between the tissues: calculation of whole-body ascorbic acid contents

The tissue ascorbate values obtained in Expt 1 permitted the calculation (Table 2) of the ascorbate contribution by, and distribution between, the individual tissues and, from this, a calculation of whole-body ascorbic acid content. The latter made use of all the partial data sets for the relevant sub-groups by assuming that there was a constant ascorbate concentration ratio between the tissues for each sub-group. The 1A1 series was chosen as being representative of the lowest overall ascorbate intake, and the dietary ascorbate values are given as the means for the entire (4-weeks) duration of the experiment.

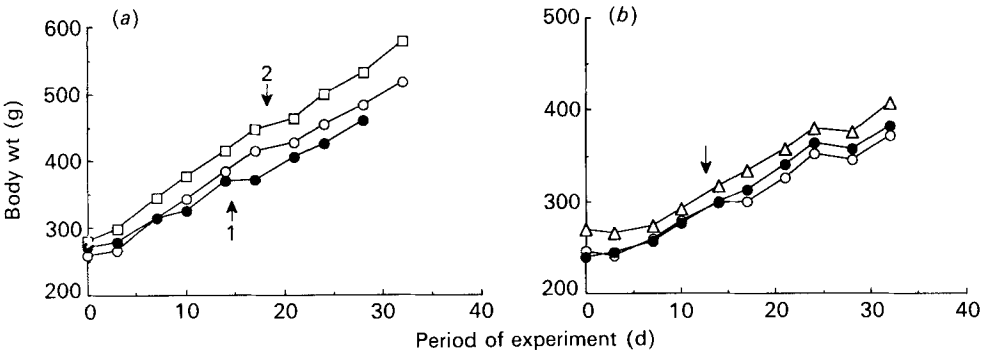

Fig. 1. Expts 1 and 2. The body-weights of guinea-pigs given graded predose and either graded post-dose or constant low post-dose levels of dietary ascorbic acid with (*a*) a relatively low dose or (*b*) a higher dose of [^{14}C]ascorbic acid. For group 1 sub-groups, in which all the animals were transferred to the low ascorbate intake immediately after ^{14}C dosing, the values have been combined into one curve for each experiment (●). For group 2 sub-groups, in which the animals were still maintained on the graded intakes of ascorbate after ^{14}C dosing, separate sub-group curves are depicted for 1A2 (□), 1E2 (○), 2B2 (△) and 2E2 (○). The remainder of the sub-groups lay between the curves that are shown and have been omitted for clarity of presentation. ↑, The point at which the ^{14}C doses were given; in Expt 1 there was a 4 d interval between dosing for groups 1 and 2, whereas in Expt 2, all the animals were dosed on the same day. All the animals were killed 13 d after dosing. For details of dietary treatments, see Table 1.

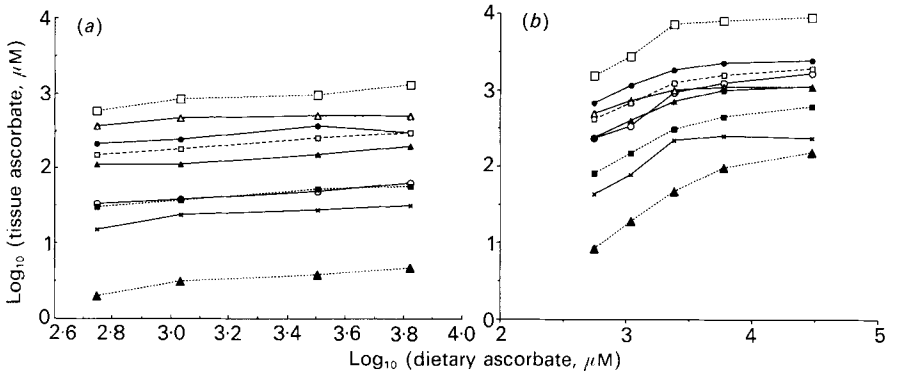

Fig. 2. Expt 1. Mean ascorbate concentrations at different sites (μM) *v.* dietary ascorbate concentration (μM) (before transfer to the low-ascorbate diet) for guinea-pigs given graded predose levels of dietary ascorbic acid and (*a*) transferred to the low dietary ascorbate level 13 d before being killed (group 1) or (*b*) maintained on constant graded dietary ascorbate levels throughout the study (group 2). (□), Adrenals; (△), brain; (●), spleen; (□), lung; (▲), eye; (■), kidney; (○), liver; (×), muscle, bone, skin, gut; (▲), plasma.

Clearly the largest contribution to the whole-body ascorbic acid content was that from muscle plus bones (mean contribution 42·2% of the total), followed by liver (23%), and then skin (17·7%; Table 2). The brain contribution rose less steeply with increasing ascorbate intake than did most other tissues, where the plasma contribution increased more steeply (see also Figs. 2 and 3).

Assessment from Expt 2 of the retention of radioactivity in undegraded ascorbic acid

Metaphosphoric acid extracts were combined in pairs from similarly treated animals and were analysed by HPLC as described previously (see pp. 719–720). This was successfully carried out for extracts from group 1 animals and from group 2 subsets A–C. In most cases, subsets D and E tissue extracts contained too little ^{14}C to be analysed effectively in this way.

Table 3 shows the mean percentage recovery during chromatography of the applied

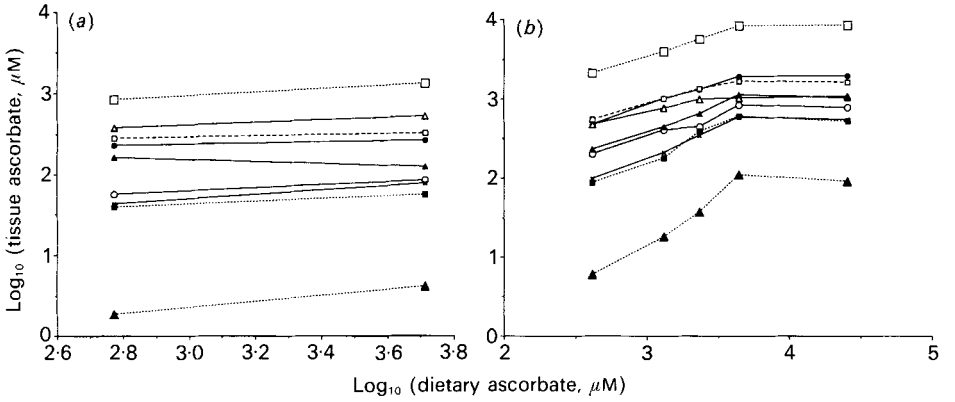

Fig. 3. Expt 2. Mean ascorbate concentrations at different sites (μM) *v.* dietary ascorbate concentration (μM) (before transfer to the low-ascorbate diet) for guinea-pigs given graded predose levels of dietary ascorbic acid and (*a*) transferred to the low dietary ascorbate level 13 d before being killed (group 1) or (*b*) maintained on constant graded ascorbate dietary levels throughout the study (group 2). (□), adrenals; (△), brain; (□), lung; (●), spleen; (▲), eye; (○), liver; (×), gut; (■), kidney; (▲), plasma.

Table 2. *Expt 1. Total body ascorbic acid content and percentage contribution from specific organs of guinea-pigs given graded predose levels of dietary ascorbic acid and either graded (sub-groups 1A2–1E2) or a constant low post-dose dietary level of ascorbic acid (sub-group 1A1)**

	Ascorbic acid (μmol/kg body-wt)						
Treatment sub-group...	1A1	1A2	1B2	1C2	1D2	1E2	Overall mean percentage contribution‡
Dietary ascorbate† (μmol/kg)...	349	545	1080	2780	6250	30700	
Tissues analysed							
Adrenals	0·19	0·45	1·13	3·30	3·27	2·95	1·06
Spleen	0·49	1·42	2·37	4·32	4·16	4·07	2·02
Liver	1·96	12·41	12·40	51·20	70·30	89·50	23·00
Kidneys	0·23	0·69	1·24	2·46	3·62	4·80	1·25
Lungs	0·84	2·21	3·86	6·48	7·70	10·82	2·50
Brain	2·90	3·21	5·04	7·04	8·03	8·45	5·09
Eyes	0·20	0·37	0·63	1·21	1·71	1·87	0·68
Intestine	1·45	2·38	3·95	6·91	6·47	5·48	3·83
Muscle and bones	7·60	22·95	41·20	112·40	127·30	126·70	42·20
Blood plasma	0·08	0·29	0·66	1·73	3·62	5·86	0·90
Skin	2·50	8·08	17·70	55·00	61·20	54·80	17·70
Whole body§	18·4	54·4	97·2	252·0	297·4	315·3	100

* For details of dietary treatments and procedures, see Table 1 and pp. 718–720.
† Mean value.
‡ For all six sub-groups: mean percentage of whole-body ascorbic acid.
§ Only one animal in each group had contributed to the analysis of all eleven tissues; for the remainder, the analyses were confined to eight tissues (adrenals, spleen, liver, kidneys, lungs, brain, eyes and blood plasma). Whole-body ascorbate was then calculated on the assumption that within each diet group, the distribution ratio of ascorbate between organs was constant. This permitted the use of all the partial data-sets for calculation of a mean value for whole-body ascorbate for all animals in each sub-group.

radioactivity for each tissue where this analysis was performed, the mean percentage which coincided with the electrochemical ascorbate peak, and the ratio, specific activity in each 'purified' fraction:apparent specific activity calculated from the total radioactivity (disintegrations/min; dpm) and total ascorbate present in the unfractionated extract.

Table 3. *Expt 2. Recovery and distribution of radioactivity during high-pressure liquid chromatographic separation of metaphosphoric acid extracts* of the labelled tissues of guinea-pigs given graded predose levels of dietary ascorbic acid and either graded (group 2) or constant low post-dose (group 1) dietary levels of ascorbic acid†*

(Mean values with their standard errors for the no. of animals shown)

Tissue	Percentage recovery of injected radioactivity			Percentage of recovered radioactivity in fraction 4‡			Specific activity ratio for column fraction 4: unfractioned extract§		
	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*
Brain	85·8	3·9	5	89·1	1·4	5	0·91	0·08	5
Eyes	102·2	1·9	5	86·2	2·5	5	1·08	0·15	5
Adrenals	90·4	2·4	6	90·0	2·4	6	1·06	0·30	6
Lungs	100·7	3·3	5	71·2	4·7	5	1·14	0·20	5
Spleen	91·3	1·6	3	59·3	5·7	3	1·02	0·07	3
Liver	101·6	4·6	5	63·3	5·3	5	0·87	0·09	5
Plasma	138·0	3·3	5	55·9	4·4	5	‖		
Overall mean (seven sites)	101·4	6·6	7	73·6	5·5	7	1·01	0·04	6

* Generally group 1 and group 2 (subgroups A–C), depending on whether sufficient radioactivity was present for detection after fractionation.

† For details of dietary treatments and procedures, see Table 1 and pp. 718–720.

‡ Fraction 4 was the fraction which contained all the electrochemically active ascorbate peak.

§ Ascorbate measured by the Cobas assay (for details, see p. 719).

‖ Ascorbate concentration too low to re-assay after chromatographic separation.

First, about 95% of the applied label was recovered in all five fractions (a total of 35–40 ml). Of this recovered material, 62–90% coincided with the ascorbate marker peak. For those three tissues (lung, spleen, liver) where the recovery in fraction 4 (the ascorbate peak) was less than 80% of the total, most of the remainder eluted at a position consistent with its being dehydroascorbate. The overall mean specific activity of the purified (fraction 4) material was very similar to the apparent specific activity of the ascorbate in the crude unfractionated extract.

Specific activities of ascorbic acid in individual tissues

Fig. 4 shows the specific activities of the ascorbic acid for combined sub-groups of group 1 (Fig. 4(*a*)) and for each of the sub-groups of group 2 (Fig. 4(*b* and *c*)), in the two experiments. These values were obtained by dividing the total metaphosphoric acid-extracted radioactivity by the total ascorbate content of the extracts, as measured by the Cobas ascorbate assay. (This calculation appeared justifiable because the apparent specific activities in the crude extracts were nearly identical with those of the column-purified ascorbate.)

The effect of varying ascorbate intakes before the [^{14}C]ascorbate dose (group 1, Fig. 4(*a*)) was quite consistent between the two experiments, but was comparatively small; moreover, all the tissues exhibited essentially the same specific activities, as indicated by the small SE, despite the very wide differences in total ascorbate concentrations (and total radioactivity (dpm)) between the different tissues.

From Fig. 4(*b* and *c*), the large gradient in unlabelled ascorbate intakes during the post-dose period in group 2 animals had a profound effect on label retention and, hence, the specific activities of tissue ascorbate at death throughout the entire range of intakes. It did not even reach a plateau between sub-groups D and E, in contrast with total ascorbate

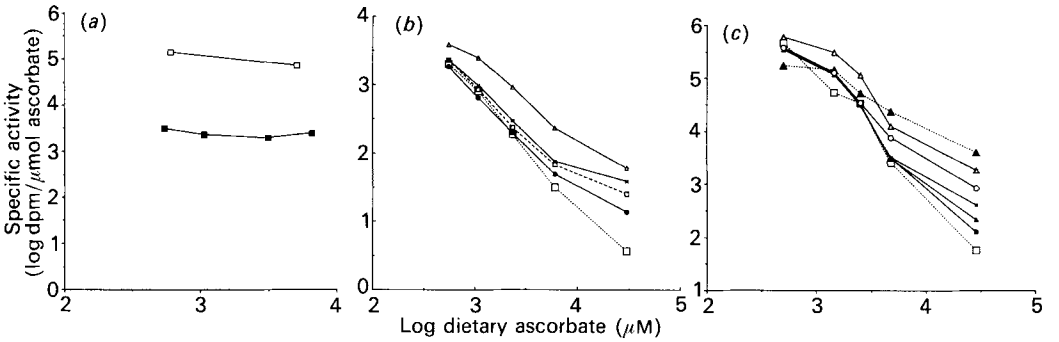

Fig. 4. Specific activities of ascorbic acid in the tissues of guinea-pigs given graded levels of dietary ascorbic acid and then either (*a*) transferred to the low dietary ascorbate levels 13 d before being killed, or (*b*, *c*) maintained on constant graded ascorbate dietary concentrations throughout the study. In (*a*) group 1 animals are shown for Expt 1; (■), a mean of seven tissues, and for Expt 2; (□), a mean of nine tissues. Inter-tissue standard errors varied between 0·02 and 0·05 (mean 0·038) on the log scale and thus lay within the symbols. In (*b*) Expt 1, and (*c*) Expt 2, the symbols denote: (△), brain; (▲), eyes; (×), muscle, bone, skin, intestine; (□), lung; (○), liver; (●), spleen; (□), adrenals; (■), plasma. For clarity, some tissues have been omitted from each figure segment. For details of dietary treatments, see Table 1.

concentrations. Some of the apparent 'fanning out' of the curves at the highest ascorbate intakes is probably an artifact of the very low levels of residual radioactivity in these extracts, especially in Expt 1. Specific activities in the brain extracts were consistently higher than those of most other tissues in both experiments, indicating a more efficient retention of label in the brain during the flush-out period. The same phenomenon was observed with the 'residual' tissues, which include skin, muscle and bone.

Urine radioactivity excretion after dosing

Fig. 5 shows the time-course of radioactivity appearing in the urines of the animals in the principal groups of the two experiments. As expected, the amount of radioactivity per mg creatinine decreased with time as the labelled ascorbate in the tissues was either degraded or was flushed out. The decline was steeper in those animals with the greater dietary ascorbate intakes than in those with the smaller intakes. The coefficient of variation for duplicate animals in this experiment was 8% for the mean of the tissue specific activities and 12% for the urine specific activity, on a creatinine basis (Fig. 6). It is not known how much of this variation was due to biological variation *per se* and how much was due to inaccuracy in dosing the animals with [^{14}C]ascorbate, or to variations in food intake plus degradation of the vitamin before ingestion. Despite these uncertainties, the magnitude of the variation was sufficiently small to encourage the future application to human studies, where the range of usual intakes can vary over an order of magnitude or more, especially if vitamin supplements are used.

When the radioactivity (per unit creatinine) in the urine of Expt 2, day 11 was plotted *v.* dietary ascorbate, or *v.* the mean specific activity of ascorbate in the tissues at death (Fig. 6), there appeared to exist a biphasic relationship, which was different at low than at high ascorbate intakes. Nevertheless, it should be possible to predict both the intake and the tissue specific activity from the urine radioactivity, over a wide range of intakes.

Analysis of the material in the urine by the Cobas ascorbate assay and by HPLC separation of ascorbate with electrochemical detection indicated that the majority of the urinary radioactivity was in compounds other than ascorbic acid. Only in groups D and E

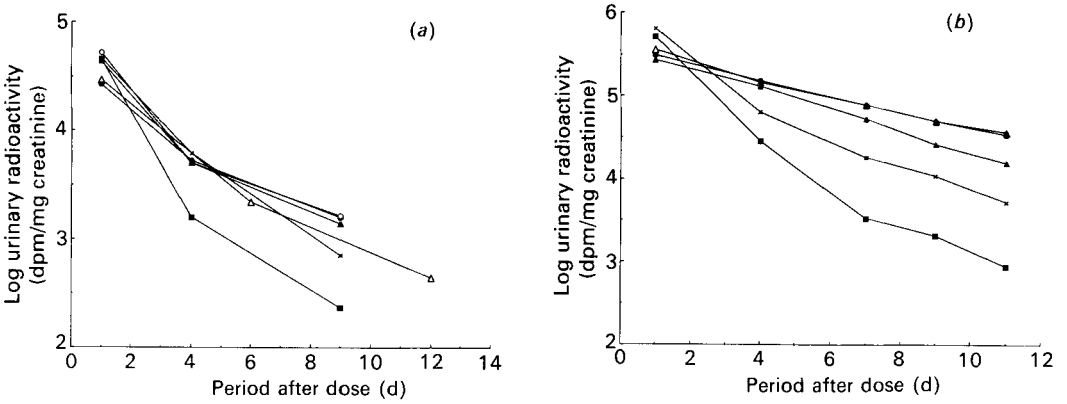

Fig. 5. Time-course of urinary excretion of ^{14}C radioactivity by guinea-pigs given graded predose levels of dietary ascorbic acid and transferred to the low dietary ascorbate levels 13 d before being killed (group 1) or maintained on constant graded ascorbate dietary levels throughout the study (group 2). (*a*) Expt 1 (relatively low dose of [^{14}C]ascorbate) (*b*) Expt 2 (higher dose of [^{14}C]ascorbate). (△), Groups 1A1, 2A1; (●), groups 1A2, 2A2; (○), groups 1B2, 2B2; (▲), groups 1C2, 2C2; (×), groups 1D2, 2D2; (■), groups 1E2, 2E2. Each point is the mean value for urine samples obtained at intervals after dosing, and is expressed as total radioactivity (disintegrations/min (dpm) per mg creatinine) on a logarithmic scale. Group 2B2 has been omitted for clarity. For details of treatment sub-groups, see Table 1.

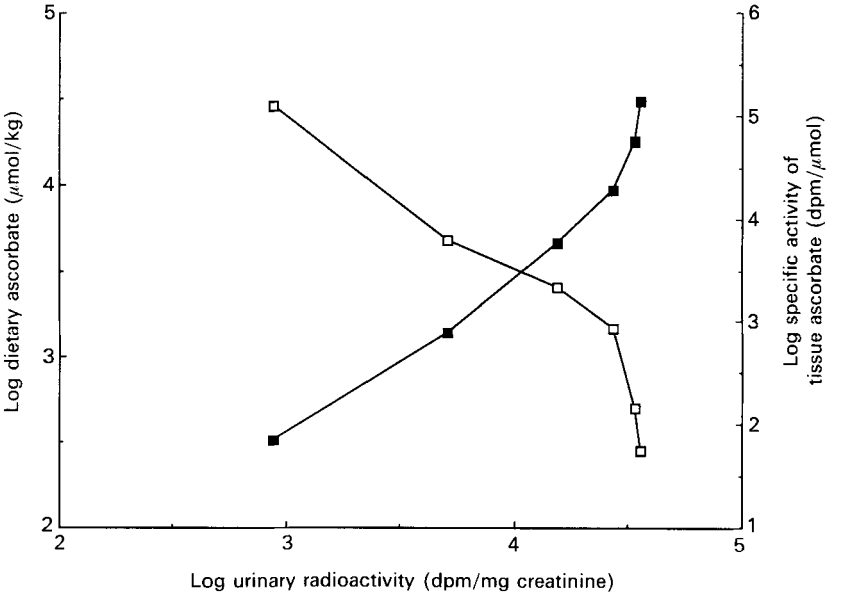

Fig. 6. Expt 2. Relationships between urinary excretion of ^{14}C radioactivity and either dietary ascorbate (□), or mean tissue ascorbate specific activity (■) on day 13 of guinea-pigs given graded predose levels of dietary ascorbic acid and graded or constant low post-dose levels of dietary ascorbic acid. For details of dietary treatment groups, see Table 1. For each index, the $\log_{10}$ of the means of the assayed values were used. For the specific activity of tissue ascorbate this was the $\log_{10}$ (mean specific activity derived from eight tissues: (disintegrations/min (dpm) per μmol)). The eight tissues included were: adrenals, eyes, spleen, lung, kidney, liver, intestine and plasma; however, brain was excluded. For the specific activity of urinary material this was $\log_{10}$ (mean dpm/mg creatinine) for the urine samples collected on day 11. Coefficient of variation of duplicates (two animals) for the urine specific activity 12%. Coefficient of variation of duplicates for the tissue specific activities 8%.

of each experiment could significant amounts of unchanged ascorbate be detected. Between 30 and 37% of the radioactivity could be precipitated by lead acetate, and part, at least, of the material yielded a peak coincident with oxalate, in an HPLC separation.

DISCUSSION

While a considerable number of previously published studies of guinea-pig ascorbate economy have estimated body pool sizes and turnover rates, generally from rates of appearance of excreted ascorbate degradation products after administration of [^{14}C]ascorbate label, few have attempted to explore the effect of different dietary ascorbate intakes on the rate of decay of this label within specific tissues, or in the body as a whole. Those studies which have estimated the total body pool size of ascorbate (Salomon, 1957; Ginter *et al.* 1971; Hornig & Hartmann, 1982) have generally obtained estimates similar to ours, after taking account of any variations in the dietary ascorbate intakes. Previous investigations have emphasized the fact that during the first few hours or days after the administration of labelled ascorbate to guinea-pigs the rate of catabolism to $^{14}CO_2$ is relatively high, and may vary considerably between individual animals for reasons possibly connected with stress (Burns *et al.* 1956; Tillotson, 1980) or with differences in age or dehydroascorbate lactonase activities (Klain *et al.* 1980; Zloch & Ginter, 1988).

Our study has confirmed the observations of Hornig & Hartmann (1982) and other earlier studies insofar that brain ascorbate label is lost less rapidly than the label from most other sites, after equilibration between the organs has been achieved. We did not observe any evidence of major differences in turnover between the other organs examined, but in the absence of a time-course this conclusion remains tentative.

Our observations on the nature of the label remaining in the tissues, 13 d after dosing, are consistent with the conclusions of Burns *et al.* (1951); Salomon (1957); Hornig *et al.* (1974) and others, that the majority of label retained in the body is probably in unchanged ascorbic acid. It seems likely that partial degradation, responsible for labelled material other than ascorbate during HPLC separation, may have arisen by interaction with oxidizing sites on the column. During the course of the present study it was found that the inclusion of homocysteine (100 μg/ml) in the metaphosphoric acid extracts of tissues could help to maintain extracted ascorbate in the reduced form and, thus, to prevent its oxidation during passage through the column; this modification is recommended for future studies. Purification of the extracted ascorbate by HPLC did not, however, significantly alter the specific activity when compared with the estimates from total tissue ascorbate and radioactivity (dpm). Within any single diet group, tissues apart from brain all had very similar specific activities at the time when the animals were killed.

The conclusion reached by Abt & von Schuching (1961), that the usual dietary intake of ascorbate may be a strong determinant of long-term turnover rate of labelled ascorbate in tissues, was clearly confirmed, and has been further extended by our own study. Abt & von Schuching (1961) made observations at three levels of intake, and concentrated mainly on whole-body kinetics. Our study has extended this to six levels of intake, spanning a wide physiological range, and has examined nine tissue sites in detail. In contrast to one of the conclusions of Abt & von Schuching (1961), the dietary intake after the labelling dose was more critical than the body pool size at the time of labelling, but this will clearly vary with the duration of the post-dose sampling period. Our observations on the urinary excretion of ^{14}C label suggest that the material in urine is a complex mixture, mainly of degradation products (Burns *et al.* 1951), but it seems to follow the same decay pattern as tissue ascorbate. It also exhibits a characteristic relation to ascorbate intakes. It may, therefore, prove useful as a proxy for the specific activities of ascorbate in tissues and, hence, for prediction of intakes or bioavailability of the vitamin.

As expected, graded intakes of ascorbic acid by guinea-pigs yielded tissue ascorbate concentrations which were strongly related to intake over the low-to-medium range, but approached an upper plateau at dietary levels above 1 g/kg diet. When a single dose of labelled ascorbate was given, 13 d before killing, the amount of the label that was retained in the tissues was dependent mainly on the amount of unlabelled ascorbate present in the subsequent diet. Thus, the amount present in the diet subsequent to the labelled dose was much more critical in determining label retention than either the amount present in the diet before the dose, or the tissue ascorbate concentration at the time of labelling. The relationship between post-dose intakes and tissue ascorbate specific activities extended over the entire range of intakes, including the interval between 1 and 5 g/kg diet, despite the fact that the tissue ascorbate levels has already reached a plateau by 1 g/kg. Thus, the entry and exchange of dietary ascorbate with tissue ascorbate must remain considerable, even when net retention within the animal is small. This implies that the specific activity of tissue ascorbate some time after a single dose of labelled vitamin may be a potentially useful index of: (*a*) ascorbate intake, given a constant bioavailability from the food sources, or (*b*) ascorbate bioavailability, given a constant rate of intake.

These indices may be of potential use in human studies which seek to measure the ascorbate intakes of free-living individuals, either to validate traditional diet-assessment techniques or to measure the biological availability of ascorbate from foods or supplements. In order to be of practical use for human studies it will be necessary to consider the use of stable isotope-labelled material such as deuterated or ^{13}C-labelled ascorbate. Two major logistic difficulties hitherto associated with such studies are: (*a*) the necessity to purify body fluid ascorbate (or a derivative of it), in order to measure the label enrichment, and (*b*) the necessity to use large and, therefore, expensive doses of the labelled vitamin, in order to obtain an adequate enrichment of the small amounts of ascorbate that can be isolated from blood or urine samples (Atkins *et al.* 1964). The results of the present study suggest that it may be possible to obtain useful data on ascorbate intake and bioavailability without necessarily isolating it from the body fluids being sampled.

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