Long-term vitamin status and dietary intake of healthy elderly subjects

2. Vitamin C

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(*Received* 15 *November* 1978 – *Accepted* 12 *February* 1979)

1. Long-term clinical and biochemical vitamin C (ascorbic acid and dehydroascorbic acid) status and dietary intake of vitamin C were monitored for 18 months in twenty-three relatively-healthy elderly subjects living at home in the north of England.

2. Plasma vitamin C showed a strong positive correlation with buffy-coat vitamin C both cross-sectionally between subjects and longitudinally within subjects; plasma levels, therefore, were almost as good an index of long-term status as buffy-coat levels.

3. Vitamin C intake was strongly correlated with plasma and with buffy-coat levels both between subjects and within subjects. This contrasts with the poor correlation observed between riboflavin intake and biochemical riboflavin status in the same subjects.

4. Subjects with relatively high average intakes showed considerable seasonal variation, and several widely-spaced measurements would be needed to characterize their long-term status accurately. Those with low average intakes and blood levels showed little variation during the study.

5. The strongest within-subject correlation was obtained by relating the biochemical values to the 7 d dietary intake directly preceding the biochemical analysis. Blood and tissue levels therefore appear to be strongly related to the current dietary intake.

6. Although some subjects had blood vitamin C levels consistently within the region associated with biochemical deficiency, none showed clinical evidence of specific deficiency symptoms.

7. After the main study, fifteen of the subjects received supplementary vitamin C for 2 months. Plasma and buffy-coat levels rose sharply, but fell to presupplementation levels within 1 month of withdrawal, emphasizing the transitory nature of increased tissue levels. No significant changes were detected in the following collagen-related urinary ratios: hydroxyproline:creatinine, proline:creatinine, proline:total amino-nitrogen and proline:hydroxyproline in hydrolysates either of whole urine or of various fractions. These variables thus appear to be insensitive to short-term changes in vitamin C status over the ranges encountered in this study.

Numerous recent studies have called attention to low blood levels of vitamin C (ascorbic acid and dehydroascorbic acid) in the elderly, but the evidence on their functional and clinical significance is conflicting (Arthur *et al.* 1967; Brocklehurst *et al.* 1968; Andrews *et al.* 1969; Berry & Darke, 1972; Department of Health and Social Security (DHSS), 1972; Eddy, 1972; MacLeod, 1972; Wilson *et al.* 1973; Eddy & Taylor, 1977).

Although several studies of elderly subjects have demonstrated a significant cross-sectional correlation between dietary intake and blood levels of vitamin C (Morgan *et al.* 1955; Roderuck *et al.* 1958; Woodhill, 1970; Burr *et al.* 1974; McLennan *et al.* 1975; McLean *et al.* 1976), there is little information available about the long-term relationships. From the practical viewpoint, it is not clear how long a period of dietary assessment is needed (*a*)

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to obtain the best correlation with biochemical indices of vitamin C status, and (*b*) to define the intake typical of a particular individual.

The present study was designed to explore the long-term relationships between dietary intake, biochemical status and clinical symptoms for several vitamins in a group of twenty-three relatively-healthy elderly subjects living at home (Rutishauser *et al.* 1979); this paper deals specifically with vitamin C.

Abnormal peptide excretion patterns have been observed during experimental human vitamin C deficiency (Burkley, 1968; Efron *et al.* 1968), and a preliminary urinary peptide analysis on the subjects in the present study (Bates, 1977) revealed a negative correlation between blood vitamin C levels and the urinary ratio, proline: total amino-nitrogen. Some of the subjects, therefore, were given vitamin C (ascorbic acid) supplements for 2 months after the main study, and the changes in vitamin C levels and urinary peptide excretion patterns were studied.

METHODS

The selection of the subjects and background to the study have been described elsewhere (Rutishauser *et al.* 1979). Each subject attended the geriatric out-patient department of the local hospital six times at 12-week intervals; at each visit they received a full clinical examination, and samples of blood were taken for biochemical investigations.

Dietary

Each subject kept a daily qualitative record (menu) of all food and drink taken for 12 months, and at intervals of 6–8 weeks a weighed record was obtained to provide information on portion sizes. The collection and analysis of the dietary records has been described elsewhere (Rutishauser *et al.* 1979). Vitamin C intakes were calculated from the DHSS food composition tables (DHSS, unpublished results) with the following modifications for the group, or for individuals, as appropriate. Analyses of vitamin C were performed on a number of fruit squash drinks and canned vegetables, and also on vegetables cooked in the subjects' own homes. After extraction in metaphosphoric acid (20 g/l) in a Colworth Stomacher (Seward Laboratory, UAC House, Blackfriars Road, London SE1 9UG), the vitamin C content of the filtrate was analysed by the method described later for blood samples. The known seasonal variation in vitamin C content of potatoes was taken into account, and for subjects who habitually used heated or sterilized milk, the vitamin C content was assumed to be 50% lower than the food table value for unheated pasteurized milk. The contribution of any vitamin C supplements taken was added to the dietary intake; none of the subjects received a vitamin C supplement for any prolonged period during the main study.

Biochemical

Within 1 h of collecting the blood samples, the buffy-coat layers were separated from four 3·0 ml portions by the method of Denson & Bowers (1961) using Dextran T500 (12 g/l; Pharmacia), to sediment the red cells. Two-thirds of the upper layer were removed and mixed; the leucocytes in 0·5 ml were counted with a Coulter Counter (model D) and the remainder was centrifuged at 4° for 20 min at 2400 *g*. The white cell pellets were extracted with 1·0 ml of a solution containing metaphosphoric acid (20 g/l) and ethanol (330 ml/l).

Plasma was obtained from an 8·0 ml sample of blood as described previously (Rutishauser *et al.* 1979) and three 1·0 ml portions were mixed with equal volumes of a solution containing metaphosphoric acid (40 g/l) and ethanol (660 ml/l) these components being mixed immediately before use to minimize phase separation. All samples were stored at −25° for a few days before analysis; no detectable losses of vitamin C occurred during storage of control samples in this manner. After removal of protein by centrifugation, the vitamin C content of the

supernatant fractions was measured by a modification of Pelletier's (1968) assay, which is designed to eliminate most potential interfering substances, including 2,3-diketogulonic acid. To avoid N flushing, sulphuric acid (660 ml/l) was used instead of a nitric acid: phosphoric acid mixture for the final step, and adequate sensitivity was achieved by working on a micro scale and using 20 mm cuvettes. The blank correction varied between approximately 50% of the sample value in samples with very low vitamin C levels, and approximately 10% in samples with high levels.

Vitamin C supplementation study

Of the twenty-three subjects in the main study, sixteen (eleven men and five women) agreed to participate in the supplementation trial; one man dropped out at an early stage because of mild dyspepsia on taking the tablets. Each subject was asked to take a vitamin C tablet every day at lunch time: for the first 4 weeks (October–November) this was 100 mg, and for the second 4 weeks (November–December) it was 500 mg. The number of tablets taken was checked by counting those remaining. The supplement was discontinued after the second 4-week period. Blood samples (20 ml) were collected around 10.00 hours at the end of each 4-week supplementation period, and again 4 weeks after discontinuing the supplement. Plasma and buffy-coat vitamin C levels were measured as described previously. Morning urine samples were collected at frequent intervals (for details, see Table 6).

Total proline and total hydroxyproline in urine were measured as described previously (Bates, 1977); creatinine was measured by the procedure of Technicon Instruments Co. Ltd (1972); total amino-N by an automated modification of Goodwin's (1968) assay, and hydroxylysine in acid-hydrolysates of acetone precipitates was measured according to Blumenkrantz & Asboe-Hansen (1975).

It was not feasible to control gelatin intake, but examination of dietary records suggested that it was probably not a major contributor to urinary hydroxyproline.

In addition to the analysis of hydrolysates of whole urine, measurements of proline and hydroxyproline were also made on hydrolysates of the acetone-insoluble precipitates. Samples were treated with 5 vol. acetone, and the insoluble fraction was either hydrolysed directly, or was treated with 20 μg highly-purified Clostridial collagenase (lot no. 1486407; Boehringer) in 2·0 ml of a buffer, pH 7·6, containing (mmol/l): Tris-chloride 10, calcium chloride 5 and sodium azide 1·5. After 24 h at 37° the collagenase-resistant material was removed by a second precipitation with 5 vol. acetone, and the acetone-soluble fraction was evaporated, hydrolysed in 6 M-hydrochloric acid and analysed as described previously (Bates, 1977).

The study was approved by the ethical committees of the Dunn Nutrition Unit, Sunderland Hospital and Sunderland Local Medical Association and by the British Medical Association.

RESULTS

Main study

1. *Clinical and biochemical.* The main clinical and routine biochemical observations have been described elsewhere (Rutishauser *et al.* 1979); no sheet haemorrhages, petechiae or inflamed gums were noted in any of the subjects during the course of the study.

Table 1 shows the plasma vitamin C levels; the over-all mean value for the women was higher than for the men, but the difference was not significant. Ten subjects had mean values below 11 μmol/l (2·0 mg/l): they had low levels throughout. Subjects with relatively high mean levels showed more variation. The mid-winter values for 1975–6 were significantly lower than the summer and autumn values for 1976 ($P < 0{\cdot}01$, paired t test, between clinics 3 and 6 and between clinics 2 and 5).

Table 1. *Individual and mean values for plasma vitamin C (ascorbic acid and dehydroascorbic acid) in healthy elderly men and women**

	Plasma vitamin C (μmol/l) for individual clinics†							
Clinic no. ...	1	2	3	4	5	6	7	
Subject no.	July–Aug. 1975	Sept.–Nov. 1975	Dec.–Feb. 1975–6	Mar.–May 1976	June–July 1976	Aug.–Oct. 1976	Nov.–Dec. 1976	Mean for all six clinics
Men								
1	45·5	37·0	19·9	36·4	54·0	19·9	—	35·4
2	1·7	8·0	3·4	2·3	5·7	4·5	—	4·3
3	42·0	27·8	36·4	58·0	47·7	35·8	—	41·3
4	5·1	4·0	2·8	2·3	4·5	4·0	—	3·8
5	17·6	7·4	34·6	17·6	13·1	28·4	—	19·8
6	2·8	5·1	5·7	2·8	10·8	5·7	—	5·5
7	15·4	10·2	8·5	46·7	15·9	10·8	—	17·9
8	20·5	9·1	6·8	4·0	14·8	21·0	—	12·7
9	2·8	4·5	7·4	2·3	3·4	5·7	—	4·3
10	9·1	7·9	4·0	3·4	6·8	5·1	—	6·0
11	7·4	10·8	5·7	5·1	11·3	8·5	—	8·1
12	11·9	4·5	6·2	6·8	7·4	5·7	—	7·1
Mean	15·1	11·4	11·7	15·6	16·3	12·9	—	14·7
Women								
13	48·9	13·6	10·8	11·4	23·3	19·9	—	21·3
14	48·3	77·2	38·1	41·5	85·8	72·0	—	60·5
15	69·3	54·5	54·5	64·8	89·7	83·5	—	69·4
16	1·7	1·1	2·8	3·4	19·9	4·5	—	5·6
17	—	18·2	27·3	12·5	13·1	30·8	12·5	19·1
18	—	4·0	2·8	4·5	5·7	7·9	3·4	4·7
19	67·6	13·1	5·1	10·2	21·6	14·8	—	22·1
20	—	28·4	29·0	35·8	46·6	50·5	23·3	35·6
21	52·2	33·0	25·6	33·5	29·0	28·4	—	33·6
22	—	5·7	7·4	8·5	11·3	9·6	6·2	8·1
23	13·6	33·0	40·3	27·2	22·2	48·7	—	30·8
Mean	—	25·6	22·2	23·0	33·5	33·7	—	28·3
Mean (all subjects)	—	18·2	16·7	19·2	24·5	22·9	—	20·7

* For details, see Rutishauser *et al.* (1979).
† Clinics were held at intervals of 4 weeks, and eight subjects were seen at each clinic.

Table 2 shows the buffy-coat vitamin C levels; ten subjects had mean values below 85 nmol/10^8 cells (15 μg/10^8 cells). The only significant seasonal variation was a difference ($P < 0.05$) between clinic 4 (spring) and clinic 5 (summer). Fig. 1 illustrates the strong positive between-subject correlation between the mean plasma and mean buffy-coat vitamin C values (r +0·89, linear, +0·95, after logarithmic transformation; $P < 0.001$). A similar between-subject correlation was observed at each clinic. There was also a strong within-subject correlation (r +0·42, 115 df. $P < 0.001$) over all clinics.

Table 2 also gives the mean leucocyte count for each subject; there was a significant negative between-subject correlation between leucocyte numbers and buffy-coat vitamin C expressed per 10^8 leucocytes (r −0·65, $P < 0.001$). The correlation between leucocyte numbers and plasma vitamin C was weaker but just significant (r −0·43, $P < 0.05$). Unexpectedly a significant negative correlation (r −0·53 to −0·69, $P < 0.01$) was also observed between either plasma or buffy-coat vitamin C levels, and each of the following inter-related variables: erythrocyte numbers, total haemoglobin concentration and packed cell volume.

2. *Dietary.* Table 3 shows the mean daily intake of vitamin C over the whole study period, calculated from the mean daily intakes in each completed week of study. There was

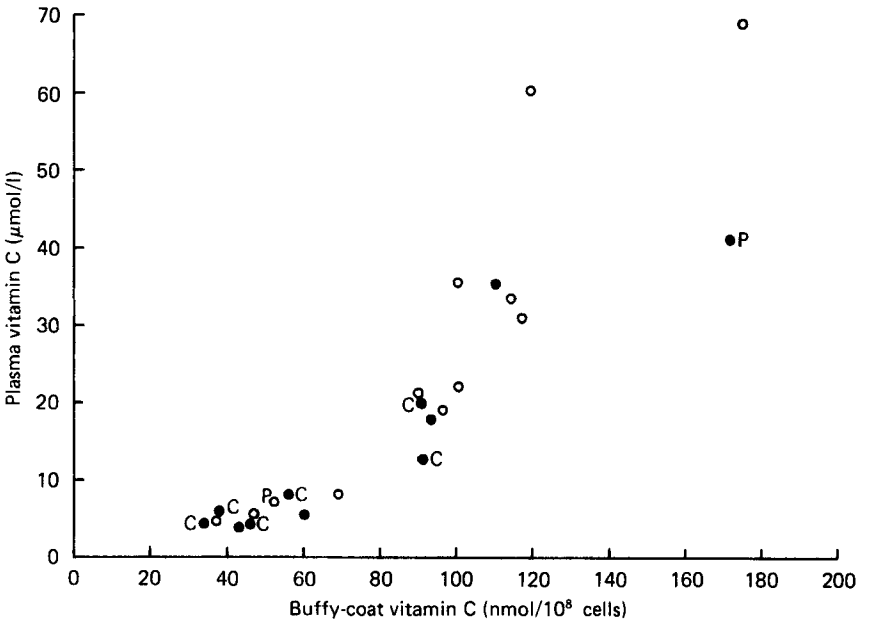

Fig. 1. Between-subject relation between plasma vitamin C (ascorbic acid and dehydroascorbic acid; μmol/l) and buffy-coat vitamin C (nmol/10^8 cells) in healthy elderly men and women (for details, see Rutishauser *et al.* 1979). ●, Men; ○, women; C, cigarette smoker; P, pipe smoker.

no difference between the over-all mean intakes of men and women; nor was there any significant seasonal variation. Six subjects had mean intakes of 25 mg or less; their plasma vitamin C levels were also very low (less than 11 μmol/l (20 mg/l) over the whole period).

The extent of the variation in vitamin C intake from week to week depended on the absolute level of intake; individuals with a low average intake showed less variation, as indicated by a smaller standard deviation, than those with high intakes. On the other hand the coefficient of variation was of the same order of magnitude over the whole range of intakes. In view of the wide variations in vitamin C intakes with time, the correlations between plasma or buffy-coat vitamin C and differing periods of dietary assessment were compared, and are shown in Table 4. Clearly the correlations between subjects were little affected by the duration of the dietary assessment but the correlations within subjects were quite markedly affected. The strongest within-subject correlation was obtained with dietary information for 1 week immediately preceding the biochemical measurements, and this was significant ($P < 0.01$) for both plasma and buffy-coat vitamin C levels. In contrast, a 1-week dietary assessment made after the clinic, which would not have contributed to the body stores at the time of blood sampling, was very poorly correlated with plasma and buffy-coat vitamin C levels within subjects (Table 4: second week after each clinic).

As the strongest within-subject correlations were obtained with dietary assessment for 1 week immediately preceding the clinic, these intakes are also shown in Table 3. On the other hand, the long-term characteristic intake is probably best obtained from the average of information for all weeks, and this is the value which is used in the further analysis of cross-sectional relationships.

Fig. 2 shows the between-subject correlation between mean daily intake (all complete weeks) and mean plasma vitamin C (all clinics) ($r+0.63$, 22 df, $P < 0.001$). The correlation was significant for the men ($r+0.81$, $P < 0.001$) but not the women ($r+0.56$, $P > 0.05$) and the smokers did not differ markedly from the non-smokers, except for one man (subject

Table 2. *Individual and mean values for buffy-coat vitamin C (ascorbic acid and dehydroascorbic acid) in healthy elderly men and women**

	Buffy-coat vitamin C (nmol/10^8 cells) for individual clinics†								
Clinic no. ...	1	2	3	4	5	6	7	Mean	Mean
Subject no.	July–Aug. 1975	Sept.–Nov. 1975	Dec.–Feb. 1975–6	Mar.–May 1976	June–July 1976	Aug.–Oct. 1976	Nov.–Dec. 1976	for all six clinics	leucocyte count ($\times 10^3$/mm^3)
Men									
1	104	124	113	106	113	98	—	110	6·75
2	52	55	44	29	55	40	—	46	10·81
3	127	189	174	206	160	173	—	172	6·05
4	60	51	32	41	35	38	—	43	7·02
5	115	67	116	87	81	80	—	91	7·27
6	62	72	66	42	67	48	—	60	7·05
7	153	53	84	87	122	60	—	93	6·38
8	109	145	73	50	75	96	—	91	4·76
9	53	33	26	18	38	38	—	34	8·58
10	60	43	23	29	47	27	—	38	8·98
11	80	78	48	49	33	50	—	56	7·18
12	72	41	52	58	57	34	—	52	10·26
Mean	87	79	71	67	74	65	—	74	7·59
Women									
13	123	43	86	78	115	88	—	90	6·95
14	107	141	118	122	119	105	—	119	6·40
15	253	134	158	157	189	157	—	175	4·76
16	62	72	31	36	—	36	—	47	6·10
17	—	111	125	68	82	106	79	96	7·38
18	—	47	51	30	32	42	19	37	7·43
19	159	69	43	95	137	97	—	100	5·87
20	—	70	88	110	128	101	111	101	5·82
21	171	114	120	97	98	100	—	117	6·38
22	—	56	72	64	80	55	85	69	6·58
23	103	90	120	132	111	146	—	117	10·26
Mean	—	86	92	90	103	94	—	97	6·72
Mean (all subjects)	—	83	81	78	87	79	—	85	7·17

* For details, see Rutishauser *et al.* (1979).
† Clinics were held at intervals of 4 weeks, and eight subjects were seen at each clinic.

no. 9) who had a very low plasma and buffy-coat vitamin C level in relation to his intake. The between-subject correlation between mean dietary intake and mean buffy-coat vitamin C (Fig. 3) was similar ($r+0{\cdot}73$, $P < 0{\cdot}001$); this is consistent with the strong positive correlation between plasma and buffy-coat vitamin C levels. The correlation was again stronger for the men ($r+0{\cdot}76$, $P < 0{\cdot}01$) than for the women ($r+0{\cdot}69$, $P < 0{\cdot}02$). Linear regression analysis indicated a barely significant difference in the intake: plasma vitamin C relation between the men and the women (t 2·0, 19 df, P *c.* 0·05), and there was no significant sex difference for the intake *v.* buffy-coat vitamin C relationship.

Supplementation study

Table 5 shows the effect of supplementation on plasma and buffy-coat vitamin C levels. By the end of the second month of supplementation (500 mg/d) every subject had substantially higher levels than before supplementation and the spread was much narrower (most subjects had, in fact responded very substantially to the first month's supplement of

Table 3. *Mean daily vitamin C (ascorbic acid and dehydroascorbic acid) (mg/d) intake from major food sources by healthy elderly men and women**

	Vitamin C intake† for the week before each clinic					Vitamin C intake‡ for all complete weeks of dietary assessment			
Clinic no. ... / Subject no.	2	3	4	5	6	Mean	SD	CV	No. of weeks
Men									
1	50	33	50	76	32	57	17	30	53
2	34	41	24	19	—	30	10	33	51
3	68	—	55†	59	—	68	42	62	18
4	19	8	13	22	—	16	6	38	48
5	26	86†	42	51	—	32	9	28	51
6	38	24	25	29	—	28	6	21	46
7	30	30	86	24	—	32	14	44	55
8	30	15	26	23	23	33	18	55	52
9	51	40	31	69	—	51	13	25	51
10	31	19	20	28	—	21	6	29	47
11	13	17	10	15	—	13	4	31	50
12	25	15	33	10	—	25	7	28	46
Mean	35	30	35	35	—	34	13	35	47
Women									
13	35	46	60	85	—	68	19	28	51
14	44	31	30	48	—	36	9	25	45
15	62	47	—	101	—	62	11	18	36
16	4	4	17	—	—	10	5	50	38
17	—	45	24	39	45	36	10	28	44
18	—	33	25	30	15	22	6	27	49
19	24	17	18	45	—	27	10	37	50
20	—	28	32	57	51	44	10	23	44
21	35	63	67	52	—	56	14	25	42
22	—	28	16	60	37	32	11	34	47
23	30	47	34	32	35	33	20	61	47
Mean	33	35	32	55	—	39	12	32	45
Mean (all subjects)	34	33	34	44	—	36	13	34	46

CV, coefficient of variation.
* For details, see Rutishauser *et al.* (1979).
† Including supplements.
‡ Excluding supplements. Subjects nos. 3, 5, 19 and 23 received occasional supplements, contributing 10, 5, 7 and 3 mg vitamin C/d over the whole study respectively.

100 mg/d, but since the results were incomplete, they have been omitted from the table). Those subjects with the lowest presupplementation levels tended to remain at the lower end during supplementation, but the difference between the 'high' and 'low' groups was much less marked. At 4 weeks after withdrawing the supplement, both plasma and buffy-coat levels had, on average, fallen to presupplementation levels.

Table 6 shows the ratios; urinary hydroxyproline:creatinine and urinary proline:total amino-N before, during and after supplementation; the subjects have been divided into two groups: those with presupplementation buffy-coat vitamin C levels of less than 80 nmol/10^8 cells (14 μg/10^8 cells) and those with presupplementation levels above this value. Neither group showed any significant change in values for these urinary ratios at any time during supplementation, and the same conclusion applies to the other urinary ratios measured (i.e. proline:creatinine, total amino-N:creatinine and proline:hydroxyproline in whole

Table 4. *Within- and between-subject correlations between intake and plasma or buffy-coat vitamin C levels in healthy elderly men and women**

(Variation in df in the within-subject correlation is due to variation in nos. of time-points for which complete dietary information was available. The between-subject correlations represent the average of all available (3–5) time-points for each subject)

			Intake *v.* plasma vitamin C		Intake *v.* buffy-coat vitamin C	
Source of correlation	Duration of dietary assessment	df	*r*	Statistical significance *P*	*r*	Statistical significance *P*
Within subjects	Day before each clinic	67	+0·42	< 0·001	+0·05	NS
	3 d before each clinic	66	+0·57	< 0·001	+0·33	< 0·01
	7 d before each clinic	69	+0·62	< 0·001	+0·41	< 0·001
	4 weeks before each clinic	81	+0·57	< 0·001	+0·29	< 0·01
	12 weeks before each clinic	88	+0·22	< 0·05	+0·08	NS
	Second week after each clinic	64	+0·26	< 0·05	+0·06	NS
Between subjects	Day before each clinic	22	+0·65	< 0·001	+0·72	< 0·001
	3 d before each clinic	22	+0·71	< 0·001	+0·73	< 0·001
	7 d before each clinic	22	+0·66	< 0·001	+0·71	< 0·001
	4 weeks before each clinic	22	+0·59	< 0·01	+0·70	< 0·001
	12 weeks before each clinic	22	+0·59	< 0·01	+0·70	< 0·001
	All available days (1·5 year period)	22	+0·62	< 0·001	+0·69	< 0·001
	Second week after each clinic	22	+0·60	< 0·001	+0·77	< 0·001

NS, not significant.
* For details, see Rutishauser *et al.* (1979).

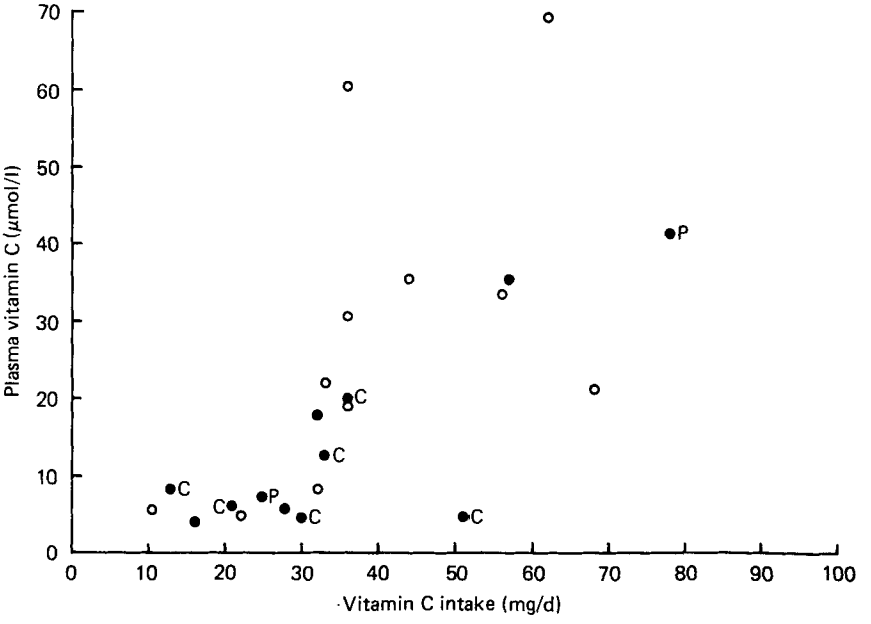

Fig. 2. Between-subject relation between plasma vitamin C (ascorbic acid and dehydroascorbic acid; μmol/l) and vitamin C intake (mg/d) in healthy elderly men and women (for details, see Rutishauser *et al.* 1979). ●, Men; ○, women; C, cigarette smoker; P, pipe smoker.

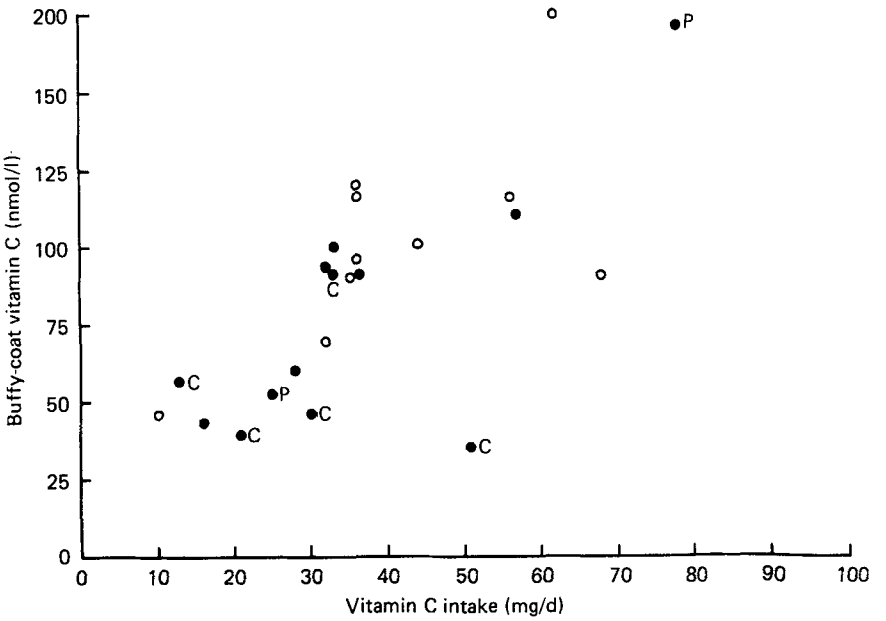

Fig. 3. Between-subject relation between buffy-coat vitamin C (nmol/10^8 cells) and vitamin C intake (mg/d) in healthy elderly men and women (for details, see Rutishauser *et al.* 1979). ●, Men; ○, women; C, cigarette smoker; P, pipe smoker.

Table 5. *Effect of vitamin C supplementation for 2 months, followed by withdrawal of supplement for 1 month, on plasma and buffy-coat vitamin C levels in healthy elderly men and women**

Subject no.	Plasma vitamin C (μmol/l)			Buffy-coat vitamin C (nmol/10^8 cells)		
Men	A	B	C	A	B	C
1	35·2	58·0	24·4	110	142	85
2	4·2	23·8	2·3	46	116	44
5	19·9	56·3	19·9	91	157	78
6	5·5	37·0	10·8	60	143	70
7	17·6	76·0	15·9	93	180	66
8	12·5	84·5	19·3	91	213	91
9	4·4	68·8	5·7	34	118	27
10	6·1	71·5	9·7	38	116	50
11	8·0	82·4	12·5	56	178	71
12	7·1	38·1	11·4	52	146	49
Women						
13	21·0	54·0	11·4	89	141	63
14	60·2	89·3	40·4	119	155	78
16	5·6	69·4	11·3	47	157	53
21	33·6	74·5	23·3	116	155	82
23	30·8	69·8	25·6	117	184	101
Mean (all subjects)	18·1	63·5	16·3	77	153	67
SD	16·0	18·9	9·5	31	27	20
Paired t (*v.* A)		−9·3	0·9		−10·2	2·2
Statistical significance: *P*		< <0·001	NS		< <0·001	*c.* 0·05

A, Average of six analyses over 18 months at 3-month intervals, before supplementation; B, value after 2 months supplementation; C, value 1 month after withdrawal of supplement.

* For details, see Rutishauser *et al.* (1979).

Table 6. *Effect of supplementation for 2 months, followed by withdrawal of supplementation for 1 month, on urinary molar ratios, hydroxyproline:creatinine and proline:total amino-nitrogen in healthy elderly men and women**

		Hydroxyproline:creatinine ($\times 10^3$)						Proline:total amino-nitrogen ($\times 10^3$)					
Period ...		(a)	(b)	(c)	(d)	(e)	(f)	(a)	(b)	(c)	(d)	(e)	(f)
Subject no.													
Group A:	Sex												
2	♂	21·5	23·6	19·2	20·4	24·0	22·2	27·3	24·3	26·6	24·7	24·9	26·6
6	♂	14·0	11·3	11·5	12·5	11·0	10·4	23·8	24·6	21·9	21·9	22·3	21·1
9	♂	15·5	19·4	22·5	18·0	16·0	18·0	27·5	27·3	26·1	25·8	25·3	27·6
10	♂	17·5	16·6	15·7	16·2	13·9	12·8	30·0	33·2	33·2	34·1	30·6	31·4
11	♂	13·3	19·2	15·6	16·0	12·8	14·7	17·4	20·1	19·0	20·2	18·8	21·1
12	♂	36·0	35·5	35·4	25·5	29·4	31·6	19·4	20·5	19·9	19·2	18·2	27·6
16	♀	13·5	14·2	14·0	12·2	14·4	16·3	24·5	23·2	23·8	20·5	21·3	22·7
Mean		18·7	20·0	19·1	17·3	17·4	18·0	24·3	24·7	24·4	23·8	23·1	25·4
SD		8·1	7·9	8·0	4·6	6·8	7·1	4·5	4·5	4·8	5·2	4·3	3·9
Paired *t* *v.* period (a)		—	−1·1	−0·3	0·9	1·2	0·6	—	−0·6	−0·1	0·4	1·9	−0·8
Group B:													
1	♂	19·3	22·4	22·0	22·2	25·3	24·3	18·3	18·3	15·2	19·2	18·2	17·3
5	♂	23·8	19·8	13·8	17·2	12·6	12·1	22·5	19·7	18·8	21·2	20·4	21·8
7	♂	18·5	23·5	22·1	25·6	24·0	25·8	25·0	24·3	24·7	23·8	27·7	22·1
8	♂	14·8	15·7	16·9	14·2	14·9	14·6	19·9	22·1	22·9	24·9	27·8	22·4
13	♀	23·8	22·2	25·4	30·8	28·5	23·4	24·6	25·2	25·2	26·0	23·8	21·3
14	♀	13·7	—	16·2	17·5	15·6	17·0	23·3	—	22·8	21·4	21·2	19·8
21	♀	44·4	38·4	30·8	31·7	28·2	29·0	27·0	26·2	28·3	23·8	25·3	25·9
23	♀	19·2	23·5	23·3	14·6	18·2	20·8	15·6	16·4	18·7	16·5	17·7	14·6
Mean		22·1	22·6	21·4	21·7	20·9	20·9	22·0	21·9	22·1	22·1	22·8	20·6
SD		9·7	7·2	5·5	7·0	6·3	5·9	3·8	3·5	4·2	3·2	4·0	3·4
Paired t *v.* period (a)		—	−0·1	0·6	0·4	0·4	0·5	—	0·1	−0·1	0·1	−0·6	2·0

Group A, subjects with mean buffy-coat vitamin C levels < 80 nmol/10^8 cells before supplementation; group B, subjects with mean buffy-coat vitamin C levels > 80 nmol/10^8 cells before supplementation; period: (a), mean of three samples on successive days just before supplementation; (b), mean of three samples during the first week after starting the 100 mg/d vitamin C supplement; (c), mean of three samples during weeks 2–4 of this supplement; (d), mean of three samples during the first week after starting the 500 mg/d supplement; (e), mean of three samples during weeks 2–4 of this supplement; (f), mean of three samples during the 2 weeks following withdrawal of the supplement.

* For details, see Rutishauser *et al.* (1979).

urinary hydrolysates; hydroxylysine:creatinine and hydroxyproline:creatinine in hydrolysates of acetone precipitates, and proline:hydroxyproline in collagenase digests of the acetone precipitates). Each subject maintained remarkably constant values for the urinary ratios during the period of measurement, between-subject variation being considerably greater than within-subject variation.

DISCUSSION

The strong between-subject correlation between plasma and buffy-coat vitamin C levels has been found in other studies on elderly subjects (Griffiths *et al.* 1967; Andrews & Brook, 1968; Loh & Wilson, 1971*a*; Burr *et al.* 1974). In the present study the between-subject correlation was strong at each clinic, and the within-subject correlation was also highly significant (the lower correlation coefficient for the within-subject correlation probably reflects the smaller range of intakes within subjects). These correlations imply a stable relationship between plasma and buffy-coat levels; moreover, the plasma level generally gave the same assessment of status as the buffy-coat level. Measurement of plasma level, which requires less blood and is easier to perform is thus likely to be adequate to identify individuals 'at risk', although the extent of tissue desaturation at the lower end is probably better defined by the buffy-coat level.

A further problem in interpreting buffy-coat levels stems from the negative correlation between these values and the total leucocyte count, noted here and in previous studies (Loh & Wilson, 1971*b*; McLennan & Hamilton, 1976; Marchand & Pelletier, 1977). A variable amount, usually approximately half, of the buffy-coat vitamin C is in the platelets (Barkhan & Howard, 1958; Gibson *et al.* 1966; Attwood *et al.* 1974), and since the ratio, platelet: leucocyte count can vary widely between individuals (in the present study the range was 19–87), it has been argued that because the platelets are not included in the calculation, buffy-coat vitamin C expressed per 10^8 cells can be misleading. The alternative calculation of buffy-coat vitamin C expressed per ml blood (Griffiths, 1968), however, did not result in a closer correlation with plasma vitamin C nor with dietary intake in the present study.

The findings of the present study suggest also that a single measurement of plasma or buffy-coat level is adequate to identify individuals with low tissue levels since they had low biochemical values at every clinic. Depending on the 'cut-off' point chosen, some individuals with intermediate mean levels would sometimes be classified as 'at risk' and sometimes not, so that several widely-spaced measurements would be needed to characterize their long-term status. Subjects with low plasma levels in the 1972–3 DHSS survey (DHSS, 1979) tended to have low mean values in the present study and there was a moderate correlation between the two sets of values ($r+0{\cdot}43$, $P < 0{\cdot}05$). Buffy-coat levels, on the other hand, showed no correlation with the corresponding values in the 1972–3 survey ($r+0{\cdot}18$, not significant); the reason for this is not clear.

Although the long-term characteristic intake of vitamin C is not likely to be accurately assessed in a single week's measurement, subjects with an habitually very low intake (less than 20 mg/d) would be correctly identified as being possibly at risk in a single week's measurement because of their relatively small variation of intakes. Some individuals with a higher average intake would occasionally fall below the at-risk level, but since the genuinely at-risk individuals are correctly identified both from short-term dietary measurements and plasma vitamin C levels, single short-term dietary measurements are meaningful in cross-sectional studies.

The strong between-subject correlations between dietary intake and blood levels have been found in other studies (Morgan *et al.* 1955; Morse *et al.* 1956; Roderuck *et al.* 1958;

Woodhill, 1970; Burr *et. al.* 1974; McLennan *et al.* 1975; McLean *et al.* 1976) and indicate that biochemical status is strongly influenced by dietary intake. This is confirmed by the within-subject correlations, which show that variations in intake are followed quite rapidly by corresponding changes in blood, and presumably also in tissue, levels. Since the changes in buffy-coat vitamin C did not correlate significantly with changes in intake between successive 3-month periods, and correlated best with changes in intake for the week before each clinic (Table 4), it seems likely that tissue levels usually responded within 1 week to changes in intake. The rapid response of blood levels to changes in intake is further emphasized by the supplementation study.

Vitamin C levels rose rapidly during supplementation, as has been noted in other studies of the elderly (Kirk & Chieffi, 1953; Bowers & Kubik, 1965; Griffiths *et al.* 1967; Andrews *et al.* 1969; Burr *et al.* 1975). However, the rapidity with which the levels fell back after withdrawal was also very striking (cf. Kirk & Chieffi, 1953). Clearly it would be necessary to give frequent supplements in order to maintain blood levels, and presumably body stores, at the levels normally found in younger people.

This rapid response of blood levels to changes in intake also means that, in studying relationships between diet and biochemistry, the timing of blood sampling relative to the dietary information is of considerable importance. Non-coincidence of blood-sampling and dietary measurement would obscure a genuinely existing correlation, because individuals show considerable seasonal and other non-recurring variations in intake: this seems to apply particularly to those subjects with a fairly good long-term status. Although dietary information for 1 week directly preceding the biochemical measurement gave the best within-subject correlations between diet and biochemistry, it is possible that an even shorter period of dietary assessment would have resulted in a stronger correlation, especially between diet and plasma levels, if the dietary assessment was sufficiently accurate over the shorter period. In the present study, variations in food portion size were not taken into account as the dietary calculations were based on a single portion size for each subject, and this would introduce some error in short-term assessment of intake. In addition, assessment of dietary vitamin C using food table values is likely to be less accurate over a short time period due to the wide range of vitamin C concentrations occurring in each type of food, and the further variable losses which occur during processing, cooking and storage.

The functional significance of low blood vitamin C levels has proved difficult to delineate. Specific clinical symptoms of vitamin C deficiency were not detected in any of the subjects who might, on biochemical grounds, have been expected to show them. Clearly therefore, low biochemical values can persist for long periods in elderly people without precipitating overt deficiency symptoms.

Attempts to assess functional significance by measurement of urinary products of collagen turnover have also proved unrewarding. The negative correlation between blood vitamin C levels and the urinary ratio, proline:total amino-N which was observed in the earlier stages of the present study (Bates, 1977) was not matched by a decrease during supplementation and is therefore unlikely to provide a sensitive functional index of deficiency. Likewise, the hydroxyproline:creatinine value, which responded positively to supplementation in the study of Windsor & Williams (1970), failed to do so in the present study. Indeed, the hydroxyproline:creatinine values were within the range found in our laboratory in young subjects with high blood vitamin C levels, and low vitamin C levels do not appear to be associated with low hydroxyproline:creatinine values. Burkley (1968) and Efron *et al.* (1968) observed a slight increase in the hydroxyproline:creatinine value during experimental vitamin C deficiency in humans, however it is clear from studies on scorbutic guinea-pigs that urinary hydroxyproline peptides are much less markedly affected than other connective tissue-related variables (Mitoma & Smith, 1960; Barnes & Kodicek,

1972; Bates, 1979). The results of the present study likewise suggest that in healthy elderly human subjects, marked changes in blood vitamin C levels can occur without any detectable changes in urinary collagen-related peptides.

First and foremost the authors with to thank the subjects for their patient co-operation over such a long period. They are indebted to the nutrition field workers, Miss E. Allen, and Mrs E. Muncaster, the nurse, Mrs K. Hodgson, the staff at the Sunderland General Hospital for help and outpatient facilities, and the staff at the Sunderland Royal Hospital for routine biochemical and haematological analyses. They thank Mrs M. Fleming, Mrs C. Godfrey, Mrs B. Sutcliffe and Mr D. R. Ashby for technical assistance, Mr K. Day for computer data analysis, and Dr R. G. Whitehead and Professor A. N. Exton-Smith for their encouragement and advice. Unpublished information from the 1967–8 and 1972–3 surveys was kindly made available to us by the Department of Health and Social Security.

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Printed in Great Britain