Selenium and vitamin E status of healthy and institutionalized elderly subjects: analysis of plasma, erythrocytes and platelets

BY DOUGLAS CAMPBELL[1], VALDA W. BUNKER[2]*, ANITA J. THOMAS[2] AND BARBARA E. CLAYTON[3]

[1] *Chemical Pathology and Human Metabolism,* [2] *University Geriatric Medicine,* [3] *Faculty of Medicine, University of Southampton, Southampton General Hospital, Tremona Road, Southampton SO9 4XY*

(*Received 26 September 1988 – Accepted 22 February 1989*)

Levels of selenium in whole blood, plasma, erythrocytes and platelets, glutathione peroxidase (*EC* 1.11.1.9; GSH-Px) activity in erythrocytes and platelets and vitamin E, low-density-lipoprotein (LDL)-cholesterol and vitamin E: LDL cholesterol in plasma were measured in seventy-five healthy subjects aged < 65 years and twenty-eight healthy and twenty-three institutionalized elderly people aged > 65 years. Healthy elderly subjects had significantly lower levels of Se in whole blood and plasma when compared with younger subjects. Other measurements of Se status were not significantly different. In the healthy subjects plasma levels of vitamin E and LDL-cholesterol increased with age to 60 years and decreased above 80 years. Vitamin E: LDL cholesterol values were not affected by age. Measurements of Se and vitamin E status in the institutionalized elderly compared with the healthy elderly were all reduced with the exception of platelet Se levels and erythrocyte GSH-Px activity. Ageing *per se* had minimal effect on Se and vitamin E status but intercurrent illness and decreased food intake can lead to reduced levels in the elderly.

Selenium: Vitamin E: Old age.

Selenium as a component of glutathione peroxidase (glutathione: hydrogen peroxide oxidoreductase, *EC* 1.11.1.9; GSH-Px) and vitamin E are important factors in the protection of membrane lipids against peroxidation, and it has been suggested that free-radical formation and lipid peroxidation with consequent membrane damage are important factors in the ageing process (Harman, 1981). Many degenerative diseases common in the elderly may also be associated with increased lipid peroxidation which might increase the requirement for antioxidants (Halliwell & Gutteridge, 1985). Thus the elderly may particularly require adequate antioxidant protection, but relatively little is known about tissue or circulating levels of Se and vitamin E and their possible interrelations in this age group.

The Se content of some animal tissues is reported to increase with age (Persigehl *et al.* 1977; Burch *et al.* 1979), but reports on the levels of Se in plasma and erythrocytes and whole blood GSH-Px activity in elderly human beings compared with younger individuals are not consistent (Thomson *et al.* 1977; Lloyd *et al.* 1983; Verlinden *et al.* 1983*a*). Whether the reported changes result from altered dietary intake or absorption of Se, underlying disease or other reasons related to ageing is unknown.

Tissue levels of Se are generally related to dietary intake (Neve *et al.* 1985), which varies considerably depending on the sources of foodstuffs in relation to the geographical distribution of Se in the soil (Burk, 1976). Hence findings from one investigation are not necessarily applicable to another and any study investigating Se status should always

* For reprints.

include an appropriately chosen 'control' group. Clinical syndromes unambiguously attributable to Se deficiency have only been reported where the intake of Se is extremely low, notably in certain parts of China and in patients receiving total parenteral nutrition. The only established function of Se in human beings is as a component of GSH-Px, but it is not known which biochemical index best reflects Se status. It has been suggested that the measurement of Se levels and GSH-Px activity in platelets might be additionally useful (Levander *et al.* 1983; Neve *et al.* 1985).

The few studies which have investigated vitamin E status in the elderly compared with younger people are contradictory, with no change (Vatassery *et al.* 1983), an increase (Lewis *et al.* 1973) or a decrease (Vandewoude & Vandewoude, 1987) being reported. Most studies have failed to extend the range examined to include a significant number of subjects aged over 70 years or take account of the possible confounding effect of plasma lipids on plasma vitamin E levels.

In an attempt to elucidate the effects of old age, rather than the effects of underlying disease, selected measurements of Se and vitamin E status including the determination of Se levels and GSH-Px activity in platelets were carried out in apparently healthy individuals aged 20–89 years. In addition the effect of chronic disease in the elderly was assessed in a group of long-stay geriatric patients.

METHODS

Subjects

Apparently healthy individuals

Seventy-five members of the hospital staff aged less than 65 years volunteered to participate. The group consisted of twenty-nine men (mean age 38·7 years, range 20–57 years) and forty-six women (mean age 38·9 years, range 20–63 years). Six men and twelve women were cigarette smokers and twenty-three men and twenty-seven women regularly drank alcohol. Seventeen men (mean age 80·7 years, range 74–89 years) and eleven women (mean age 75·9 years, range 73–79 years) living independently in the community were recruited with the help of local general practitioners. One man regularly smoked cigarettes and five men and four women regularly drank alcohol.

Institutionalized elderly

Nine men (mean age 75·4 years, range 70–85 years) and fourteen women (mean age 81·9 years, range 71–90 years) who had been resident in a long-stay geriatric hospital in Southampton for over 2 months were selected with the help of nursing and medical staff. Four men and two women were regular cigarette smokers and one man and two women drank alcohol regularly. They suffered from a variety of chronic conditions, such as cardiovascular disease, arthritis, Parkinson's disease and chronic obstructive airways disease, and were receiving various medications including diuretics, tranquillizers, antidepressants and laxatives. None of them had a diagnosis of hepatic, renal, gastrointestinal or malignant disease.

All subjects were questioned concerning their smoking and drinking habits and details recorded according to the method of Shaper *et al.* (1982).

The study was approved by the Joint Ethical Sub-Committee of the Faculty of Medicine of the University of Southampton and Southampton and South West Hampshire District Health Authority.

Analyses

Blood (15 ml) was collected into three tubes each containing EDTA as an anticoagulant. One tube was reserved for the measurement of haemoglobin (Hb), packed cell volume, GSH-Px activity and whole blood Se concentration. The remainder of the blood was

Table 1. *Details of analyses carried out on whole blood, plasma and platelets of subjects aged > 65 years and < 65 years*

Measurement	Medium	Between-batch precision (%)	Technique	Reference
Selenium	Plasma	4·1	Hydride-generation atomic absorption spectrophotometry	Lloyd *et al.* (1982)
	Whole blood	4·6		
	Platelet	5·9		
Glutathione peroxidase (*EC* 1.11.1.9)	Whole blood	7·4	Kinetic assay at 37° using *t*-butylhydroperoxide as substrate	Beutler (1979)
	Platelet	6·1*		
Vitamin E (α-tocopherol)	Plasma	4·0	High-performance liquid chromatography	Bieri *et al.* (1979)
Low-density lipoprotein	Plasma	3·7	Measurement of cholesterol after selective precipitation of high- and very-low-density lipoproteins	Wako Chemicals GmbH
Protein	Platelet	2·5	Automated colorimetric technique	Lowry *et al.* (1951)
Haemoglobin	Whole blood	1·5	Manual colorimetric cyanmet-haemoglobin method	Dacie *et al.* (1984)
Packed cell volume	Whole blood	1·8	Hawksley microhaematocrit centrifuge	Dacie *et al.* (1984)

* Within batch precision.

centrifuged for 15 min at 180 ***g*** to sediment erythrocytes and leucocytes. The platelet-rich plasma was removed and centrifuged for 10 min at 1100 ***g***. The resultant platelet-poor plasma was removed and stored for the analysis of plasma levels of Se, vitamin E and low-density-lipoprotein (LDL)-cholesterol. The platelet button was washed three times with phosphate buffer (pH 7·0) before being resuspended in the same buffer. The suspension was used to measure levels of Se and protein and GSH-Px activity in platelets. Details of the methods are given in Table 1. Samples for the measurement of GSH-Px activity were assayed within 48 h of collection. The concentration of Se in the erythrocytes was calculated from the levels in plasma and whole blood, and is expressed in two different ways (nmol/g Hb and μmol/l cells) to enable comparisons with published studies to be made.

Statistical evaluation

Each group of results was examined to assess their closeness to a normal distribution. All values approximated to such a distribution and the mean and 95% confidence interval (95% CI) was calculated for each analyte.

Multiple-regression analysis was used to assess the effect of sex, age and alcohol and tobacco consumption in the apprently healthy subjects. Association between variables was determined using Pearson's correlation coefficient. Analysis of variance, with smoking as a covariable, was used to compare the values from the different groups.

RESULTS

Results, together with the tests of significance, are shown in Table 2.

The effects of alcohol, tobacco consumption and sex were assessed independently of age, in the healthy subjects aged < 65 years, but only smoking caused a change. Platelet GSH-Px activity (mean, 95% CI) was significantly ($P < 0·05$) reduced in the smokers (120 (95% CI 104–136) U/g protein) compared with the non-smokers (138 (95% CI 131–145) U/g

Table 2. *Values for selenium, vitamin E and glutathione peroxidase (glutathione: hydrogen-peroxide oxidoreductase, EC 1.11.1.9) for apparently healthy subjects aged < 65 years and > 65 years and institutionalized elderly patients*

	Apparently healthy					
	< 65 years		> 65 years		Institutionalized elderly	
Measurement	Mean	95% CI	Mean	95% CI	Mean	95% CI
Se						
Whole blood (μmol/l)	1·37	1·32–1·42	1·23**	1·13–1·32	1·00†††	0·93–1·07
Plasma (μmol/l)	1·20	1·15–1·25	1·03***	0·94–1·12	0·81†††	0·73–0·88
Erythrocyte: (nmol/g Hb)	4·85	4·64–5·06	4·74	4·43–5·14	4·27†	3·92–4·61
(μmol/l)	1·60	1·53–1·67	1·49	1·35–1·62	1·26†	1·16–1·37
Platelet (nmol/g protein)	27·6	26·0–29·2	26·6	24·4–28·7	26·8	22·7–30·8
Glutathione peroxidase						
Erythrocyte (U/g Hb)	24·0	22·5–25·5	24·2	21·5–26·8	21·2	18·6–23·8
Platelet (U/g protein)	134	127–141	131	114–147	99††	89–109
Vitamin E						
Plasma (μmol/l)	24·1	22·5–25·8	26·2	23·1–29·2	18·8†††	16·8–20·7
Vitamin E:LDL-cholesterol (μmol/mmol)	6·14	5·53–6·76	6·33	5·67–6·99	5·34†	4·55–6·14

95% CI, 95% confidence interval; Hb, haemoglobin; LDL, low-density lipoprotein.
Statistical significance of difference (analysis of variance with cigarette smoking as co-variable) between subjects aged > 65 years and those < 65 years: ** $P < 0.01$, *** $P < 0.001$.
Statistical significance of difference (analysis of variance with cigarette smoking as co-variable) between institutionalized elderly compared with healthy elderly: † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$.

protein). There was a tendency for Se levels in plasma, erythrocytes and platelets and erythrocyte GSH-Px activity to be reduced in smokers, but these differences did not reach statistical significance ($P > 0.05$).

The healthy elderly had significantly ($P < 0.05$) lower plasma and whole blood levels of Se when compared with subjects aged < 65 years, but the concentration of Se and GSH-Px activity in erythrocytes and platelets were unaffected by age. Although the mean plasma vitamin E and LDL-cholesterol levels of the elderly were not significantly different from those of younger subjects, there was a significant ($P < 0.025$) age-associated change in both variables which increased steadily up to about 60 years and then decreased after 80 years. Mean (95% CI) values for plasma levels of vitamin E as a function of age were 20–29 years (*n* 12) 21·5 (19·7–23·3) μmol/l; 30–39 years (*n* 21) 21·6 (19·5–23·8) μmol/l; 40–49 years (*n* 25) 25·8 (22·6–29·0) μmol/l; 50–59 years (*n* 10) 28·5 (22·4–34·6) μmol/l; 70–79 years (*n* 18) 28·0 (24·0–32·0) μmol/l; 80–89 years (*n* 9) 23·9 (19·2–28·6) μmol/l. When plasma vitamin E levels were expressed in terms of plasma LDL-cholesterol levels no age-associated change was detected.

The institutionalized elderly subjects had significantly lower levels of Se in whole blood ($P < 0.001$), plasma ($P < 0.001$) and erythrocytes ($P < 0.05$) when compared with the healthy elderly. Both groups had similar Se concentrations in platelets. The institutionalized subjects also had reduced GSH-Px activity in their platelets ($P < 0.05$) and lower plasma vitamin E levels ($P < 0.001$) and vitamin E:LDL-cholesterol ($P < 0.05$) compared with the healthy elderly subjects.

In the healthy subjects there was a significant correlation between the level of Se in plasma and erythrocytes (*r* 0·49, $P < 0.001$) and between the level of Se and GSH-Px activity in both erythrocytes (*r* 0·32, $P < 0.01$) and platelets (*r* 0·44; $P < 0.01$). Plasma levels

of vitamin E correlated with those of LDL-cholesterol (r 0·28, $P < 0·05$). There was no significant relation between the levels of vitamin E or vitamin E:LDL-cholesterol in plasma and any measurement of Se status.

DISCUSSION

Levels of Se in plasma and whole blood, but not in platelets and erythrocytes, were significantly lower in the healthy subjects aged > 65 years when compared with those aged < 65 years. The decrease in the plasma levels is in agreement with those reported by other workers (Thomson *et al.* 1977; Miller *et al.* 1983; Verlinden *et al.* 1983*a*). It may be the result of a reduced intake of protein (Bingham *et al.* 1981), since there is a significant correlation between the dietary intake of protein and Se in the elderly (Bunker *et al.* 1988), but the possibility of altered plasma binding of Se in the elderly cannot be excluded. The institutionalized elderly subjects had significantly lower plasma Se levels than the healthy elderly. Reduced dietary intake of protein in these subjects (Thomas *et al.* 1988) is likely to be an important factor, although chronic disease is also known to reduce plasma (serum) levels (Sullivan *et al.* 1979; Miller *et al.* 1983).

Erythrocyte Se levels, which are believed to reflect long-term Se status (Neve *et al.* 1985), were comparable in the healthy young and elderly subjects, suggesting that despite the possible reduced dietary Se intake of the elderly, it was nevertheless sufficient to maintain erythrocyte Se levels. Varying results have been reported by other workers (Thomson *et al.* 1977; Miller *et al.* 1983; Verlinden *et al.* 1983*a*). Valid comparisons are difficult because of differences in analytical techniques and the populations studied. The institutionalized elderly subjects had reduced erythrocyte Se levels when compared with the healthy elderly. Nutritional factors are important, but certain chronic diseases are reported to be associated with decreased erythrocyte Se concentrations (Miller *et al.* 1983).

Erythrocyte GSH-Px activity, which was measured to provide a functional index of long-term Se status, did not change with age. A significant, but weak, correlation was demonstrated in erythrocytes between the Se level and the activity of GSH-Px in the combined group of healthy young and elderly subjects. This relation appeared to persist across the range of Se levels measured without a plateau being reached and was similar to that reported by Verlinden *et al.* (1983*b*). The reduced erythrocyte Se levels in the institutionalized elderly were not reflected in the erythrocyte GSH-Px activity which was similar to that found in the healthy elderly. This observation is difficult to explain, since in the healthy subjects there was a correlation between erythrocyte Se levels and GSH-Px activity. However, the relation was rather weak, and only about 10% of human erythrocyte Se is associated with GSH-Px (Neve *et al.* 1985). Recently van Rij *et al.* (1987) also reported the maintenance of erythrocyte GSH-Px activity in subjects with low Se status.

It has been suggested (Neve *et al.* 1985) that the measurement of Se in platelets may, because of the relatively short lifetime of platelets, be useful in assessing Se status. No difference in platelet Se levels was noted between healthy young and elderly subjects. In contrast to the findings in whole blood, plasma and erythrocytes, there was no difference between the concentration of Se in the platelets from healthy and institutionalized elderly subjects. This finding is in agreement with that of Kasperek *et al.* (1982) who suggested a special role for Se in platelets due to their tendency to retain the trace element in deficiency states even when the concentration in plasma and erythrocytes is reduced. The activity of GSH-Px in platelets was unaffected by age. The institutionalized elderly, however, had significantly lower GSH-Px activity than the healthy elderly, despite both groups having similar levels of Se in their platelets. The same observation was reported by Kasperek *et al.* (1982) and Menzel *et al.* (1983) in children receiving dietetic treatment for phenylketonuria and maple syrup urine disease.

Plasma vitamin E levels in the healthy subjects rose steadily up to about 60 years of age, then decreased in those aged over 80 years. The finding that vitamin E concentrations increase with age has been reported previously (Lewis *et al.* 1973), but Wei Wo & Draper (1975) and Vandewoude & Vandewoude (1987) also demonstrated a fall in levels in subjects aged over 70–80 years. The tendency for plasma vitamin E concentrations to increase with age is not surprising, as almost 70% of the vitamin E in plasma is associated with the LDL (McCormick *et al.* 1960) which is also known to increase with increasing age (Lewis *et al.* 1974). When the plasma vitamin E levels of the healthy subjects were expressed in terms of LDL-cholesterol the age-associated change disappeared. This confirms the recent findings of Vandewoude & Vandewoude (1987). The institutionalized elderly subjects had significantly lower plasma vitamin E levels than the healthy elderly and this could not be explained by reduced levels of LDL-cholesterol, a finding in agreement with that of Kelleher & Losowsky (1978).

The results of our study suggest that ageing *per se* has little effect on Se and vitamin E status and that intercurrent illness and reduced food intake are the most important factors in the reduced status reported in the elderly.

The authors thank the volunteers and their general practitioners for permission to study their patients and Dr Reid, Dr Eastwood, Professor Hall and Professor Briggs for permission to study inpatients under their care, and Mrs D. Brown for her assistance with the preparation of the manuscript. Financial support from Research into Ageing, The Wessex Medical School Trust, the BMA Charlotte Eyck Award 1984 and the British Geriatric Society Dhole Bursary 1987 is gratefully acknowledged. V. W. B. is supported by The Health Promotion Research Trust and B. E. C. holds a Leverhulme Emeritus Fellowship.

REFERENCES

Beutler, E. (1979). Glutathione peroxidase (GSH-Px). In *A Manual of Biochemical Methods*, 2nd ed, pp. 71–73. New York: Grune and Stratton.

Bieri, J. G., Tolliver, T. J. & Catignani, G. L. (1979). Simultaneous determination of α-tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. *American Journal of Clinical Nutrition* **32**, 2143–2149.

Bingham, S., McNeil, N. I. & Cummings, J. H. (1981). The diet of individuals: a study of a randomly-chosen cross section of British adults in a Cambridgeshire village. *British Journal of Nutrition* **45**, 23–25.

Bunker, V. W., Lawson M. S., Stansfield, M. F. & Clayton, B. E. (1988). Selenium balance studies in apparently healthy and housebound elderly people eating self-selected diets. *British Journal of Nutrition* **59**, 171–180.

Burch, R. E., Sullivan, J. F., Jetton, M. M. & Hahn, H. K. J. (1979). The effect of aging on trace element content of various rat tissues. 1. Early stages of aging. *Age* **2**, 103–107.

Burk, R. F. (1976). Selenium in man. In *Trace Elements in Human Health and Disease*, pp. 105–133 [A. S. Prasad and D. Oberleas, editors]. New York: Academic Press.

Dacie, J. V. & Lewis, S. M. (1984). *Practical Haematology*, 6th ed. Edinburgh: Churchill Livingstone.

Halliwell, B. & Gutteridge, J. M. (1985). *Free Radicals in Biology and Medicine*, Oxford: Clarendon Press.

Harman, D. (1981). The aging process. *Proceedings of the National Academy of Sciences, USA* **78**, 7124–7128.

Kasperek, K., Lombeck, I., Kiem, J., Iyengar, G. V., Wang, Y. X., Feinendegen, L. E. & Bremer, H. J. (1982). Platelet selenium in children with normal and low selenium intake. *Biological Trace Element Research* **4**, 29–34.

Kelleher, J. & Losowsky, M. S. (1978). Vitamin E in the elderly. In *Tocopherol, Oxygen and Biomembranes*, pp. 311–327 [C. de Duve and O. Hayaishi, editors]. Amsterdam: Elsevier/North Holland Biomedical Press.

Levander, O. A., DeLoach, D. P., Morris, V. C. & Moser, P. B. (1983). Platelet glutathione peroxidase activity as an index of selenium status in rats. *Journal of Nutrition* **113**, 55–63.

Lewis, B., Chait, A., Wootton, I. D. P., Oakley, C. M., Krinkler, D. M., Sigurdsson, G., February, A., Maurer, B. & Birkhead, J. (1974). Frequency of risk factors for ischaemic heart-disease in a healthy British population. With particular reference to serum-lipoprotein levels. *Lancet* **i**, 141–146.

Lewis, J. S., Pian, A. K., Baer, M. T., Acosta, P. B. & Emerson, G. A. (1973). Effect of long-term ingestion of polyunsaturated fat, age, plasma cholesterol, diabetes mellitus, and supplemental tocopheral upon plasma tocopherol. *American Journal of Clinical Nutrition* **26**, 136–143.

Lloyd, B., Holt, P. & Delves, H. T. (1982). Determination of selenium in biological samples by hydride-generation and atomic absorption spectroscopy. *Analyst* **107**, 927–933.
Lloyd, B., Lloyd, R. S. & Clayton, B. (1983). Effect of smoking, alcohol, and other factors on the selenium status of a healthy population. *Journal of Epidemiology and Community Health* **37**, 213–217.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with folin phenol reagent. *Journal of Biological Chemistry* **193**, 265–275.
McCormick, E. C., Cornwell, D. G. & Brown, J. B. (1960). Studies on the distribution of tocopherol in human serum lipoproteins. *Journal of Lipid Research* **1**, 221–228.
Menzel, H., Steiner, G., Lombeck, I. & Ohnesorge, F. K. (1983). Glutathione peroxidase and glutathione S-transferase activity of platelets. *European Journal of Paediatrics* **140**, 244–247.
Miller, L., Mills, B. J., Blotcky, A. J. & Lindeman, R. D. (1983). Red blood cell and serum selenium concentrations as influenced by age and selected diseases. *Journal of the American College of Nutrition* **4**, 331–334.
Neve, J., Vertongen, F. & Molle, L. (1985). Selenium deficiency. *Clinics in Endocrinology and Metabolism* **14**, 629–656.
Persigehl, M., Schicha, H., Kasperek, K. & Klein, H. J. (1977). Trace element concentrations in human organs in dependence of age. *Beiträge zur Patholozie* **161**, 209–220.
Shaper, A. G., Pocock, S. J., Walker, M., Wale, C. J., Clayton, B., Delves, H. T. & Hinks, L. (1982). Effects of alcohol and smoking on blood lead in middle-aged British men. *British Medical Journal* **284**, 299–302.
Sullivan, J. F., Blotcky, A. J., Jetton, M. M., Hahn, H. K. J. & Burch, R. E. (1979). Serum levels of selenium, calcium, copper, magnesium, manganese and zinc in various human diseases. *Journal of Nutrition* **109**, 1432–1437.
Thomas, A. J., Bunker, V. W., Hinks, L. J., Sodha, N., Mullee, M. A. & Clayton, B. E. (1988). Energy, protein, zinc and copper status of twenty-one elderly inpatients: analysed dietary intake and biochemical indices. *British Journal of Nutrition* **59**, 181–191.
Thomson, C. D., Rea, H. M., Robinson, M. F. & Chapman, O. W. (1977). Low blood selenium concentrations and glutathione peroxidase activities in the elderly. *Proceedings of the University of Otago Medical School* **55**, 18–19.
Vandewoude, M. F. J. & Vandewoude, M. G. (1987). Vitamin E status in a normal population: the influence of age. *Journal of the American College of Nutrition* **6**, 307–311.
van Rij, A. M., Wade, C., Kirk, I., Thomson, C. & Robinson, M. F. (1987). Altered platelet lipoxygenase activity in patients with low selenium. *Clinical Science* **73**, 525–529.
Vatassery, G. T., Johnson, G. J. & Krezowski, A. M. (1983). Changes in vitamin E concentrations in human plasma and platelets with age. *Journal of the American College of Nutrition* **4**, 369–375.
Verlinden, M., van Sprundel, M., Van der Auwera, J. C. & Eylenbosch, W. J. (1983*a*). The selenium status of Belgian population groups. II. Newborns, children and the aged. *Biological Trace Element Research* **5**, 103–113.
Verlinden, M., van Sprundel, M., Van der Auwera, J. C. & Eylenbosch, W. J. (1983*b*). The selenium status of Belgian population groups. I. Healthy adults. *Biological Trace Element Research* **5**, 91–102.
Wako Chemicals GmbH. *β-Lipoprotein C Method Sheet*. Neuss, West Germany: Wako Chemicals GmbH.
Wei Wo, C. K. & Draper, H. H. (1975). Vitamin E status of Alaskan Eskimos. *American Journal of Clinical Nutrition* **28**, 808–813.

Printed in Great Britain