The effect of age and frailty upon blood esterase activities and their response to dietary supplementation

JOANNA SUMMERBELL, HILARY WYNNE, CATHERINE R. HANKEY¹ & FAITH M. WILLIAMS²

Wolfson Unit of Clinical Pharmacology, The University, Claremont Place, Newcastle upon Tyne NE2 4HH, ¹Department of Dietetics, Royal Victoria Infirmary, Queen Victoria Road, Newcastle upon Tyne NE1 4LP and ²Department of Environmental and Occupational Medicine, The University, The Medical School, Newcastle upon Tyne NE2 4HH

- 1 The aims of this study were two-fold. First, to define ranges of blood esterase activities in three groups, namely young subjects, fit community dwelling elderly and frail, chronically hospitalised elderly subjects, and second, to determine whether low blood esterase activities in the frail patients could be altered by increasing their nutritional intake.
- 2 Plasma cholinesterase, aspirin esterase, paraoxonase and phenylacetate esterase activities were all significantly lower in the frail elderly compared with the young and fit elderly volunteers. The activity of red blood cell esterase was not different in the frail elderly.
- 3 Fourteen frail elderly patients were randomly assigned to receive either hospital meal provision plus supplemental feeding with Build-up (Nestlé) and Maxijul (SHS Ltd) or hospital provision alone for 8 weeks. Dietary intake was measured for all patients at the start of the study and at week 8. Measurements of blood esterase (cholinesterase, phenylacetate esterase, paraoxonase, aspirin esterase and red blood cell esterase), albumin and anthropometric indices (weight, triceps skinfold thickness and mid arm circumference) were made before the study and repeated at week 4 and 8.
- 4 There was a significant increase in plasma cholinesterase at week 4 ($P < 0.05$) but this was not statistically significant at week 8. There were no significant changes in any of the other esterase activities or anthropometric measurements.
- 5 We conclude that the lower esterase activities of the frail chronically hospitalised elderly do not respond to dietary supplementation for a period of 8 weeks with routinely available products. The hypothesis that lower esterase activities are the direct result of undernutrition which would be corrected by dietary supplementation has not been supported by this study.

Keywords age frailty esterases nutrition

Introduction

Although of a similar chronological age, elderly individuals may differ markedly in their functional state. Those who remain independent in activities of daily living have been defined as the fit elderly, whilst those dependent on others for their care have been defined as frail [1]. Although a functional definition, biological differences between these groups have subsequently been reported. Consequences for drug metabolism include a reduced activity of plasma and serum aspirin esterase, cholinesterase, phenylacetate esterase and albumin concentration as well as drug clearance in the

frail elderly when compared with their fit counterparts [2, 3].

There is a close association between some plasma esterase activities and nutritional status. Thus, for example, a fall in cholinesterase activity has been reported in the serum and liver of fasting rodents [4] as has a positive correlation between serum cholinesterase activity and food intake in undernourished children [5]. Plasma cholinesterase and paraoxonase levels have been closely linked with lipid metabolism [6, 7]. Cholinesterase is manufactured in the liver but is not stored and

*Correspondence: Dr Hilary Wynne, Wolfson Unit of Clinical Pharmacology, The University, Claremont Place, Newcastle upon Tyne NE2 4HH

immediately enters the general circulation [8], and thus, in the absence of changes in degradation rate, any changes in the rate of synthesis by the liver are directly reflected in the plasma.

Malnourishment is prevalent in the institutionalised elderly. In a study of 196 individuals over 65 years old living either independently, in residential accommodation or sheltered housing, or in continuing-care hospitals, subclinical deficiencies including iron, ascorbic acid, vitamin D and vitamin B6 were most prevalent in the latter group [9].

The aim of this study was twofold. First, to define ranges of blood esterase activities in three groups, young healthy subjects, fit elderly subjects and frail elderly subjects. Second, to ascertain whether esterase activities in the frail elderly patients are related to nutrition by determining their response to a period of nutritional supplementation.

Methods

Twenty-two fit subjects (18 female) <25 years of age, (weight 59 ± 2 kg) mean $\pm$ s.e. mean recruited from amongst hospital and university colleagues, 20 fit elderly subjects (15 female) >65 years of age, (66 ± 2 kg), living independently at home, and recruited from local social organisations for the elderly and 20 frail elderly patients (11 female) >65 years of age, (42 ± 2 kg), receiving continuous hospital care for chronic disabling conditions such as stroke or musculo-skeletal degeneration took part. The fit elderly were independent in all activities of daily living whereas the frail elderly were chronically dependent upon nursing staff for hygiene, mobility and nutritional requirements. No subject was suffering an acute medical illness. None was receiving drugs that are known to affect plasma aspirin esterase activity [10, 11].

Informed consent was obtained from all volunteers and from the next of kin of the frail elderly subjects. The study had the approval of the Newcastle Health Authority/University Joint Ethics Committee. Venous blood samples (20 ml) were collected into lithium heparin tubes for measurements of plasma and red blood cell esterases, and into plain tubes (5 ml) for measurement of serum total protein and albumin. Plasma and red blood cells were separated and plasma stored at -80°C . Analysis was carried out within 4 weeks. The red blood cells were washed with saline and stored at -80°C and assayed within 24 h. Haematocrit was similar in the three groups of subjects. To compare our results with the earlier observations of Williams *et al.* [2], plasma aspirin esterase and cholinesterase activities in 7 young, 7 fit elderly and 20 frail elderly were measured. Paraoxonase, phenyl acetate esterase and red blood cell esterases were also measured in samples from all subjects in the three groups.

To assess the effect of dietary supplementation on esterase activities, a further 20 frail elderly patients (16 female), selected because of a plasma albumin less than 40 g l^{-1} were randomly assigned to a 'control' or 'supplemented' group in a diet supplementation study. From measurements of esterase activities in the frail elderly, we calculated that with 10 patients in each group, the study would be sufficiently sensitive to detect a 30%

change in esterase activities at an α value of 0.05 and a β value of 0.2 [12]. Having lost three subjects from each group due to the practical problems of removal to other care institutions ($n = 4$), and mortality ($n = 2$), only seven of the supplemented (five female) and seven of the control group (six female), completed the study. A change of 37% in esterase activities would have then been required to fulfil these power requirements. Members of the control group (weight 38 ± 3 kg) were offered normal hospital provision for the duration of the study (8 weeks). The supplemented group (weight 46 ± 4 kg) also received one unit of Build-up (Nestlé) (1365 kJ) twice daily and up to 2100 kJ of a glucose polymer (Maxijul, SHS Ltd) daily.

At the start of the study all the frail patients selected had a reduced plasma aspirin esterase activity compared with the lowest value previously obtained for a fit elderly population of 100 nmol salicylate produced ml^{-1} plasma min^{-1} [2].

Dietary intake was measured for all patients at the start of the study (week 0) and at week 8 using three day diaries (which included supplements in the treated group). Key nurses completed the records, and were supervised by the dietitian. Food portion size was standardized and waste collected and weighed by the dietitian. The intake, calculated from actual portion weights or the equivalent published weights for individual items [13] was determined using the Microdiet dietary analysis system [14].

Blood esterase and albumin measurements were made at weeks 0, 4 and 8 in the frail patients in the diet supplementation study. Plasma aspirin esterase was measured (at 37°C) as described by Williams *et al.* [2], plasma phenylacetate esterase (at 37°C) as described by Williams *et al.* [15] and with addition of physostigmine to inhibit plasma cholinesterase, plasma paraoxonase (at 37°C) by the method of Eckerson *et al.* [16] and plasma cholinesterase (at either 37°C or 30°C) by the method of Kalow & Lindsay [17]. A validated correction factor is available [18].

Red blood cell (RBC) esterase was measured at 37°C by a micromodification of the method described by Johnson [19] using phenylvalerate (1.4 mM) as substrate. Formation of phenol was measured by spectrophotometry at 510 nm. Total protein and albumin were measured by the hospital biochemistry service using a sequential multichannel analyser with computer (SMAC), total protein by the Biuret reaction and albumin by mixing serum with bromocresol green and measuring absorbance by spectrophotometry. The intraindividual variation in blood esterase activities measured repeatedly over a period of 9 months was 8% for plasma aspirin esterase ($n = 4$), 8% for cholinesterase ($n = 5$), 11% for paraoxonase ($n = 4$), 3% for phenyl acetate esterase ($n = 4$) and 9% for RBC esterase ($n = 4$).

Anthropometric measurements of weight, triceps skinfold thickness (TSF) and mid arm circumference (MAC) were made at weeks 0, 4 and 8 (20).

To ascertain the significance of differences between the young, fit and frail elderly subjects, one way analysis of variance (ANOVA) was used. In the supplementation study, the significance of changes within individuals was assessed by ANOVA for repeated measures followed by the Scheffe *F* test.

Results

The mean values for weight, albumin, total protein, plasma aspirin esterase, cholinesterase, paraoxonase, phenylacetate esterase and red blood cell esterase for the three groups of subjects are shown in Table 1. Plasma aspirin esterase, cholinesterase, paraoxonase and phenylacetate esterase activities were significantly ($P < 0.05$) lower in the frail elderly patients compared with the young and fit elderly volunteers when expressed per ml plasma. Enzyme activities for aspirin esterase and cholinesterase were not different from those reported previously [2]. Plasma aspirin esterase and paraoxonase activities were significantly ($P < 0.05$) lower in the frail elderly patients compared with the young and fit elderly volunteers when expressed per mg plasma protein, whilst cholinesterase activity was significantly ($P < 0.05$) lower in the frail elderly patients compared with the fit elderly

volunteers and phenylacetate esterase was significantly ($P < 0.05$) lower in the frail elderly patients compared with the young when expressed in this way. In contrast to the plasma esterases, red blood cell esterase activities were not lowered in the frail elderly. Red blood cell esterase activity was expressed in relation to red cell number but there was no difference in haematocrit between the three groups. The frail elderly patients had low mean weights compared with the fit elderly subjects and to published reference values [9, 21, 22] and low plasma albumin concentrations compared with the fit elderly and to published reference values [9, 23].

In the study to assess the effect of dietary supplementation, the calculated mean daily energy intake of supplemented patients increased from 4490 ± 330 kJ at week 0 to 7310 ± 430 kJ at week 8 ($P < 0.01$), but did not change in the control group during the same period (4880 ± 480 kJ to 4800 ± 490 kJ). Although this resulted

Table 1 Weight, albumin, and plasma and red blood cell esterase activities in frail elderly, fit elderly and young individuals. Results expressed as mean (95% CI)

	Frail elderly	Fit elderly	Young
Weight (kg)	42* (38–46)	66 (62–70)	59 (55–63)
Total protein (g l ⁻¹)	63* (61–65)	67 [×] (66–68)	71 (69–73)
Albumin (g l ⁻¹)	34* (32–36)	42 [×] (40–44)	48 (46–50)
Aspirin esterase (nmol salicylate formed ml ⁻¹ plasma min ⁻¹)	64* (56–72)	119 (99–139)	115 (95–135)
(nmol salicylate formed mg ⁻¹ protein min ⁻¹)	1.02* (0.87–1.17) <i>n</i> = 20	1.59 (1.42–1.76) <i>n</i> = 7	1.52 (1.2–1.84) <i>n</i> = 10
Cholinesterase (nmol benzoylcholine chloride hydrolysed ml plasma min ⁻¹ (37° C))	1170* (1010–1130)	1730 (1460–2000)	1670 (1400–1940)
(nmol benzoyl choline chloride hydrolysed mg ⁻¹ protein min ⁻¹)	19.7 ⁺ (17.4–22.0) <i>n</i> = 20	26.4 (22.1–30.7) <i>n</i> = 7	22.9 (19.1–26.7) <i>n</i> = 9
Phenylacetate esterase (nmol phenol formed ml ⁻¹ plasma min ⁻¹)	65* (57–73)	83 (73–93)	99 (91–107)
(nmol phenol formed mg ⁻¹ protein min ⁻¹)	1.05 [×] (0.92–1.18) <i>n</i> = 20	1.24 (1.06–1.42) <i>n</i> = 20	1.40 (1.29–1.51) <i>n</i> = 22
Paraoxonase (nmol paranitrophenol formed ml ⁻¹ plasma min ⁻¹)	100* (86–114)	146 (128–164)	144 (124–166)
(nmol paranitrophenol formed mg ⁻¹ protein min ⁻¹)	1.60* (1.38–1.82) <i>n</i> = 20	2.18 (1.87–2.49) <i>n</i> = 20	2.05 (1.83–2.27) <i>n</i> = 22
Red blood cell esterase (nmol phenol formed 10 ⁶ RBC ⁻¹ min ⁻¹)	128 (112–144) <i>n</i> = 19	126 (120–132) <i>n</i> = 20	117 (107–127) <i>n</i> = 19

*value significantly different from fit elderly and young individuals at $P < 0.05$ (ANOVA).

[×]value significantly different from young individuals at $P < 0.05$ (ANOVA).

⁺value significantly different from fit elderly individuals at $P < 0.05$ (ANOVA).

Table 2 Anthropometric measurements of weight, triceps skinfold thickness (TSF) and mid arm circumference (MAC) in control and supplemented frail elderly patients. Mean (95% CI)

Age (years)	Supplemented group			Control group		
	0 weeks	4 weeks 88 (range 85–91) (5F: 2M)	8 weeks	0 weeks	4 weeks 87 (range 70–96) (6F: 1M)	8 weeks
Weight (kg)	45.8 (39.0–52.6)	45.9 (39.1–52.7)	47.1 (40.3–53.9)	38.0 (32.9–43.1)	37.3 (32.0–42.6)	37.8 (32.0–43.6)
TSF (mm)	8.2 (6.3–10.1)	8.1 (5.2–11.0)	7.6 (5.7–9.5)	5.6 (4.3–6.9)	5.7 (4.4–7.0)	4.8 (3.8–5.8)
MAC (cm)	22.4 (20.7–24.1)	22.7 (20.8–24.6)	22.1 (20.4–23.8)	19.5 (17.2–21.8)	19.2 (16.7–21.7)	19.3 (16.9–21.7)

Table 3 Plasma and red blood cell esterase and albumin activities in the control and supplemented frail elderly patients. Mean (95% CI)

Esterase	Supplemented group			Control group		
	0 weeks	4 weeks	8 weeks	0 weeks	4 weeks	8 weeks
1 Aspirin esterase	71 (56–86)	80 (67–93)	71 (54–88)	64 (53–75)	63 (59–67)	53 (40–66)
2 Cholinesterase (30° C)	940 (730–1150)	1160* (890–1430)	990 (720–1260)	840 (670–1010)	880 (710–1050)	780 (610–950)
3 Phenyl acetate esterase	73 (59–87)	79 (65–93)	70 (61–79)	62 (44–80)	62 (46–78)	56 (44–68)
4 Paraoxonase	117 (98–136)	127 (117–137)	130 (109–151)	99 (74–124)	105 (84–126)	94 (69–119)
5 RBC esterase	120 (87–153)	143 (120–166)	128 (95–161)	146 (127–165)	137 (117–157)	130 (112–148)
6 Albumin	34 (32–36)	36 (32–40)	35 (31–39)	34 (32–36)	34 (32–36)	33 (31–35)

¹nmol salicylate formed ml⁻¹ plasma min⁻¹²nmol benzoylcholine chloride hydrolysed ml⁻¹ plasma min⁻¹³nmol phenol formed ml⁻¹ plasma min⁻¹⁴nmol paranitrophenol formed ml⁻¹ plasma min⁻¹⁵nmol phenol formed/10⁶ RBC min⁻¹⁶g l⁻¹*value significantly different from week 0 *P* < 0.05 (ANOVA).

in a change in the mean weight of the supplemented group of +1.3 kg and of the control group of -0.2 kg, in absolute terms, these were not statistically significant. We were unable to demonstrate any statistically significant change in triceps skinfold thickness and mid-arm circumference over the 8 week period (Table 2). Plasma albumin did not significantly change in the supplemented patients (Table 3). Following a 4 week period of supplemented feeding plasma cholinesterase activity showed a significant (*P* < 0.05) increase but differences were not significant at week 8. There was no significant change in activity of plasma phenylacetate esterase, paraoxonase, aspirin esterase or RBC esterase at either week 4 or week 8 (Table 3).

Discussion

Blood esterase activities provide a dynamic indication of liver function, and use of cholinesterase activities as a reflection of protein synthesis is well established [8]. The effects of some pathological states upon activities of

these enzymes has been documented previously. For example, paraoxonase activity is known to decline after a myocardial infarction [24] and cholinesterase shows reduced activity with malignant hepatic metastases [25] and in liver disease [8]. Although there are reports of similar aspirin esterase [2] and paraoxonase [26] levels in males and females, epidemiological studies have also indicated that cholinesterase activities are about 10% lower in premenopausal women than in men, but values rise in postmenopausal women to similar values to elderly men [27]. Thus, hormonal differences would not explain the higher values found in our young subjects compared with those in the frail elderly subjects. The results from the first part of our study show that activities of plasma esterases are significantly lowered by the effects of frailty or chronic hospitalisation rather than by age *per se*. Values of plasma esterases were lower in the frail elderly compared with one or both other groups, even when expressed per mg protein suggesting that the fall in esterase activity occurs not simply in parallel with the generalised fall in protein concentration, but that synthesis of these enzymes is particularly reduced. Elucida-

tion of the mechanism of these observations is important in order that therapeutic implications may be assessed.

Mean body weights of the young and the fit subjects were in the normal anthropometric range (28), but weights of the frail elderly subjects were much lower suggesting relative undernutrition of this group. An assessment of the ideal weight of the frail elderly is difficult. Weight can be related to arm span, for example, in the fit elderly [29]. However, these values cannot be measured reliably in the frail elderly and reference ranges established.

Although malnutrition of the elderly in hospital is well documented [9] attempts to improve dietary intake often have had limited success. Banerjee *et al.* [30], for example, used the supplement Complian® in an attempt to increase the nutritional intake of long-stay geriatric patients but found that they selectively reduced their intake of fat and carbohydrate. McEvoy & James [31] determined the feasibility of supplementation using Build-up on an acute geriatric unit and were able to record a significant weight increase over a 4 week period with an increase in fat rather than muscle mass in their patients. The results in our chronically hospitalised elderly are consistent with those of Banerjee *et al.* [30] and contrast with a faster weight gain of the elderly in an acute setting, in whom underlying pathological conditions were also improving [31].

No significant changes were seen in any of the blood esterase activities except for a rise in plasma cholinesterase activity at 4 weeks ($P < 0.05$) in the supplemented group which was not sustained at 8 weeks. Had the reduced

levels of cholinesterase in the frail elderly been due to nutritional factors amenable to simple dietary supplementation, a rise in the activity of this esterase, with its short half-life of 12 days, could have been expected [32]. Albumin concentration did not change over the 8 week period, a change which may have been predicted from its half-life of approximately 21 days. However, it has been well established that albumin is a more useful marker of prognosis than of nutritional status [33]. The life span of red blood cells is approximately 120 days so that it was unlikely that any change in RBC esterase activities would be seen over this time period.

In summary, we have shown that the activities of plasma esterases are not reduced in the fit elderly compared with their young counterparts. Activities are lower in the frail elderly when compared with one or both of these groups. However, the activity of red blood cell esterase is not different in the frail elderly.

We were unable to demonstrate a significant effect on the nutritional status of our frail elderly patients by dietary supplementation for 8 weeks as routinely available to the chronically hospitalised elderly. Perhaps in consequence of our failure to achieve the improvement in nutritional status of the supplemented group of frail elderly subjects which we had anticipated, esterase activities did not change in a way which would influence drug disposition. Frailty had developed in our patients over several years, so that it is possible that a longer period of dietary supplementation might have produced improvements in nutritional status together with elevated plasma esterases.

References

- Woodhouse KW, Wynne HA, Baillie S, James OFW, Rawlins MD. Who are the frail elderly? *Quart J Med* 1988; **8**: 505–506.
- Williams FM, Wynne HA, Woodhouse KW, Rawlins MD. Plasma aspirin esterase: the influence of old age and frailty. *Age Ageing* 1986; **18**: 39–42.
- Wynne HA, Cope LH, Herd B, Rawlins MD, James OFW, Woodhouse KW. The effect of age and frailty upon paracetamol clearance in man. *Age Ageing* 1990; **19**: 419–424.
- Harrison MF, Brown LM. The effects of starvation on the pseudocholinesterase activity of the liver and the serum of rats. *Biochem J* 1951; **48**: 151–154.
- Waterlow J. Liver cholinesterase in malnourished infants. *Lancet* 1950; **258**: 908–909.
- Chu MI, Fontaine P, Kutty KM, Murphy D, Red Lundran R. Cholinesterase in serum and low density lipoprotein of hyperlipidaemic patients. *Clin Chim Acta* 1978; **85**: 55–59.
- Mackness MI, Walker CH, Carlson LA. Low A-esterase activity in serum of patients with fish-eye disease. *Clin Chim Acta* 1987; **33**: 587–588.
- Brown SS, Kalow W, Pilz W, Whittaker M, Woronick CL. The plasma cholinesterase: a new perspective. *Adv clin Chem* 1981; **22**: 1–124.
- Vir SC, Love HG. Nutritional status of institutionalized and non-institutionalized aged in Belfast, Northern Ireland. *Am J clin Nutr* 1979; **32**: 1934–1947.
- Gupta JD, Gruca M, Ablett W. Effect of other drugs and chemicals on the degradation of aspirin *in vitro*: possible extrapolation to *in vivo* metabolism of aspirin. *Eur J Drug Metab Pharmacokinet* 1979; **2**: 103–108.
- Puche E, Garciade La Servana H, Motra C, Saucedo R. Serum aspirin esterase activity in epileptic patients receiving treatment with phenobarbital, phenytoin, carbamazepine and valproic acid. *Int J clin Pharmac Res* 1989; **9**: 55–58.
- Pocock SJ. *Sample size clinical trials: a practical approach*. Chichester: Wiley, 1983; 83–107.
- Department of Health. Report on health and social subjects 41 dietary reference values for food energy and nutrients for the United Kingdom. *Report of the panel on dietary reference values of the committee on medical aspects of food policy* (1991). HMSO 1992 and Social Security.
- Crawley H. *Food portion sizes*. Ministry of Agriculture, Fisheries and Food. HMSO 1988, London.
- Williams FM, Nicholson EN, Woolhouse NW, Adjepon-Yamoah KK, Rawlins MD. Activity of esterases in plasma from Ghanaian and British subjects. *Eur J clin Pharmac* 1986; **31**: 485–489.
- Eckerson HW, Wyte CM, La Du BN. The human serum paraoxonase/arylesterase polymorphism. *Am J Hum Genet* 1983; **35**: 1126–1135.
- Kalow W, Lindsay HA. A comparison of optical and manometric methods for the assay of human serum cholinesterase. *Can J Biochem Physiol* 1955; **33**: 568–574.
- King J. *The cholinesterases: Practical enzymology* London: Van Nostrand, 1965.
- Johnson MK. Improved assay of NTE for screening organophosphates for delayed neurotoxicity potential. *Arch Toxicol* 1977; **37**: 113–115.
- Durnin JGVA, Wolmersley J. Body fat assessed from total body density and its estimation from skinfold thickness:

- measurements on 481 men and women aged 16–72 years. *Br J Nutr* 1974; **32**: 77–97.
- 21 McEvoy AW, James OFW. Anthropometric indices in normal elderly subjects. *Age Ageing* 1982; **11**: 97–100.
- 22 Campbell AJ, Borrie MJ. Reference values for upper arm anthropometric measurements for a New Zealand community sample of subject aged 70 years and over. *Hum Biol* 1988; **60**: 587–596.
- 23 Veering BTH, Brum AGL, Sovereign JHM *et al.* The effect of age on serum concentrations of albumin and α -acid glycoprotein. *Br J clin Pharmac* 1990; **29**: 201–206.
- 24 McElveen J, Mackness MI, Colley M, Peard T, Warner S, Walker CH. Distribution of paraoxon hydrolytic activity in the serum of patients after myocardial infarction. *Clin Chem* 1986; **42**: 671–673.
- 25 Whetstone HJ, La Motta RV, Bellucci H, Tennant R, White BV. Studies of cholinesterase activity v serum cholinesterase in patients with carcinoma. *Ann int Med* 1960; **52**: 102–125.
- 26 Geldmacher-von Mallinckrodt M, and Diepgen TL. The human serum paraoxonase polymorphism and specificity. *Toxicol Environ Chem* 1988; **18**: 79–196.
- 27 Lepage L, Schiele F, Gueguen R, Siest G. Total cholinesterase in plasma: biological variations and reference limits. *Clin Chem* 1985; **31**: 546–550.
- 28 Garrow JS. *Treating obesity seriously, a clinical manual*. London: Churchill Livingstone, 1981.
- 29 Bassey EJ. Demispan a measure of Skeletal Size. *Ann Hum Biol* 1986; **13**: 499–502.
- 30 Banerjee AK, Brocklehurst JC, Wainwright H, Swindell R. Nutritional status of long-stay geriatric inpatients: the effects of a food supplement (Complan). *Age Ageing* 1978; **7**: 237–242.
- 31 McEvoy AW, James OFW. The effect of a dietary supplement (Build-up) on nutritional status in hospitalized elderly patients. *Hum Nutr appl Nutr* 1982; **36A**: 374–376.
- 32 Costergaard D, Viby-Mogensen J, Harel HK, Skovgaard LT. Half-life of plasma cholinesterase. *Acta Anaesthesiol Scand* 1988; **32**: 266–269.
- 33 Fleck A. Protein metabolism after surgery. *Proc Nutrition Soc* **39**: 125–132.

(Received 10 December 1992,
accepted 26 July 1993)