J Clin Pathol 1982;**35**:625-630

Value of plasma calcium, phosphate, and alkaline phosphatase measurements in the diagnosis of histological osteomalacia

HEDLEY PEACH, JULIET E COMPSTON, SHOBHANA VEDI, LEO WL HORTON*

From the Department of Community Medicine, Gastrointestinal Research Unit and Department of Histopathology, St Thomas' Hospital and Medical School, London

SUMMARY Plasma calcium and phosphate concentrations and alkaline phosphatase activities were examined retrospectively in 50 patients with histologically proven osteomalacia and 50 age- and sex-matched control subjects with normal bone histology. An abnormal plasma alkaline phosphatase activity was more useful than an abnormal plasma calcium or phosphate concentration in distinguishing between normal and osteomalacic subjects, producing a false-negative rate of 14% and a false-positive rate of 8%. False-negative and false-positive rates of 10% and 8% respectively were obtained when the presence of an abnormality in any one of the three biochemical measurements was used as a predictor of histological osteomalacia. When discriminant analysis was applied to plasma calcium, phosphate and alkaline phosphatase together a false-negative rate of 12% and a false-positive rate of 0% was obtained.

Sixty-two patients in whom a diagnosis of osteomalacia was suspected were investigated prospectively, using both single biochemical abnormalities and the classification functions derived from the discriminant analysis of all three biochemical measurements to predict the presence or absence of histological osteomalacia. Plasma alkaline phosphatase activity gave false-negative and false-positive rates of 10% and 32% respectively but was a more reliable predictor of abnormal bone histology than were plasma calcium or plasma phosphate concentrations or the presence of an abnormality in any one of the three measurements. Discriminant analysis using plasma calcium, phosphate and alkaline phosphatase together produced a false-negative rate of 16% and a false-positive rate of 10%.

We conclude that plasma alkaline phosphatase activity is the best single routine biochemical screening test for osteomalacia, although a high false-positive rate may occur. Direct discriminant analysis of plasma calcium, phosphate and alkaline phosphatase together provides a more sensitive method of detecting histological osteomalacia which should be useful in determining the prevalence of osteomalacia within high-risk populations.

Osteomalacia is characterised histologically by defective bone mineralisation producing an increase in osteoid volume and seam thickness with decreased calcification fronts and a reduced mineralisation rate. The most common clinical manifestations are bone pain and proximal muscle weakness. Biochemically, hypocalcaemia, hypophosphataemia and a raised plasma alkaline phosphatase activity may occur. However, the clinical symptoms and signs are frequently non-specific and it is usually difficult or impossible to make a definite diagnosis on the basis of the clinical features until late in the course of the disease. Similarly, the biochemical changes are variable; histological osteomalacia may be seen in patients with normal plasma calcium, phosphate and alkaline phosphatase[1 2] whilst hypocalcaemia, hypophosphataemia or a raised plasma alkaline phosphatase activity may be due to causes other than osteomalacia. The radiological changes of osteomalacia, namely pseudofractures and unhealing pathological fractures, are usually seen only in the advanced stages of the disease.

*Present address: Department of Pathology, Royal Berkshire Hospital, Reading.

Accepted for publication 30 September 1981

Because of the unreliability of clinical, biochemical and radiological changes in osteomalacia, examination of bone histology is at present the only accurate method for its diagnosis. Since osteomalacia is generalised in its distribution throughout the skeleton, biopsy from a single site is representative; in practice the iliac crest is the site most commonly used. However, bone biopsy, although safe is an invasive procedure and the processing and quantification of bone histological sections are time-consuming and require facilities that are not widely available. In addition, bone biopsy is obviously an unsuitable method of screening populations for osteomalacia.

In this study we have compared plasma calcium, phosphate and alkaline phosphatase measurements in 50 patients with osteomalacia and 50 age- and sex-matched healthy control subjects with normal bone histology and have applied discriminant analysis to these three biochemical values. Sixty-two patients with suspected osteomalacia were then studied prospectively, using both single biochemical abnormalities and the classification functions derived from the discriminant analysis to predict the presence or absence of histological osteomalacia.

Patients and methods

RETROSPECTIVE STUDY

Biochemical data were collected retrospectively on 50 consecutive patients investigated at St Thomas' Hospital between 1975 and 1979 who were found to have histologically proven osteomalacia. Patients with abnormal renal or hepatic function as assessed by plasma creatinine concentrations and conventional liver function tests were excluded; patients in whom insufficient biochemical data was available were also excluded. The age, sex, clinical features and diagnoses of these patients are summarised in Table 1. Privational osteomalacia was diagnosed when lack of exposure to ultraviolet irradiation or nutritional vitamin D deficiency, or both, were believed to be important aetiological factors and after exclusion of other causes of osteomalacia.

Fifty control subjects were selected so that the number of males and females within each five year age group exactly balanced that among the patients. The controls were otherwise healthy patients who had been admitted to the surgical wards of St Thomas' Hospital for a minor operation requiring a general anaesthetic and who gave informed consent for bone biopsy to be taken during that operation. The study was given approval by the Ethics Committee of St Thomas' Hospital.

Table 1 *Characteristics of osteomalacia and control patients in the retrospective sample*

	Osteomalacia	*Control*
Number	50	50
Sex: male	22	22
female	28	28
Age (yr): mean ± SD	46·3 ± 16·2	46·2 ± 16·4
range	19-78	18-78
Nationality: Asian	18	0
British	32	50
Bone pain	39	0
Pseudofractures or pathological fractures	14	0
Aetiology:		
Privational	18	
Malabsorption:		
Small intestinal resection	7	
Polya gastrectomy	12	
Jejunoileal bypass	9	
Coeliac disease	4	

PROSPECTIVE STUDY

The prospective sample comprised all 62 patients who were referred to the Gastroenterology Department with possible osteomalacia between 1979 and 1981, excluding those patients with abnormal renal or hepatic function. Details of these patients are given in Table 2.

Table 2 *Characteristics of the patients in the prospective sample*

	With osteomalacia	*Normal bone histology*
Number	31	31
Sex: male	10	8
female	21	23
Mean age (yr)	44·8	44·9
Nationality: Asian	16	9
British	15	22
Bone pain	25	11
Pseudofractures or pathological fractures	12	0
Aetiology of suspected bone disease:		
Privational	20	13
Hypophosphataemic	2	0
Malabsorption:		
Chronic pancreatitis	0	1
Jejunoileal bypass	3	4
Polya gastrectomy	0	7
Small intestinal resection	5	4
Crohn's disease	1	1
Coeliac disease	0	1

LABORATORY METHODS

Measurements of plasma calcium, phosphate, albumin and alkaline phosphatase were made on a Vickers M 300 analyser. The plasma calcium was corrected for the plasma albumin concentration.[3] The plasma was obtained at the time of or within one week of the bone biopsy. Reference ranges were established from a population sample of 332 healthy subjects, 215 male and 117 female, age 16-65 yr.

BONE BIOPSY

Undecalcified sections of transiliac biopsy specimens (thickness 8 μm), were quantitatively assessed using a Zeiss 25 point eye-piece graticule. Calcification fronts were assessed both from sections stained with 1% toluidine blue and by fluorescence microscopy of unstained sections after oral administration of demethylchlortetracycline, 900 mg, 48 hours before the biopsy. A histological diagnosis of osteomalacia was made when the osteoid volume (% total cancellous volume) was greater than 10% and the calcification fronts (% osteoid surface) were less than 60%. The osteoid volume was between 10 and 20% in 21 patients (mean 14·5), 21-40% in 20 patients (mean 27·4) and 41-60% in 9 patients (mean 49·1). The mean (± SD) osteoid volume and calcification fronts in the control group were 3·4 (± 2·2) and 81·4 (± 8·1) respectively.

STATISTICAL METHODS

The data were analysed by means of the Statistical Package for the Social Sciences using the subprogramme Discriminant.[4] A direct method of discriminant analysis was used in which all discriminating variables were entered directly into the analysis. The discriminant scores assigned to individual cases were plotted for patients with and without histological proof of osteomalacia to obtain the value which gave the lowest number of false-positives. The following sets of variables were entered into successive discriminant analyses: calcium; phosphate; alkaline phosphatase; calcium + phosphate + alkaline phosphatase. The variables which were found to give the most satisfactory discrimination between osteomalacic and control cases were used to derive a set of classification functions which would permit the classification of a sample of patients studied prospectively whose membership of osteomalacic and control groups was unknown.

Results

RETROSPECTIVE STUDY (FIGS 1-4, TABLE 3)

Five of the 50 patients with osteomalacia had normal plasma calcium and phosphate concentrations and alkaline phosphatase activity. In the remaining 45 patients the biochemical abnormalities were as follows: raised plasma alkaline phosphatase and hypocalcaemia (24), raised plasma alkaline phosphatase alone (18), hypocalcaemia alone (2) and hypophosphataemia and raised plasma alkaline phosphatase (1). Normal plasma calcium, phosphate and alkaline phosphatase were found in 43 of the 50 control subjects. Of the remaining seven, one had a low plasma phosphate and a raised plasma alkaline phosphatase, one had a raised corrected plasma

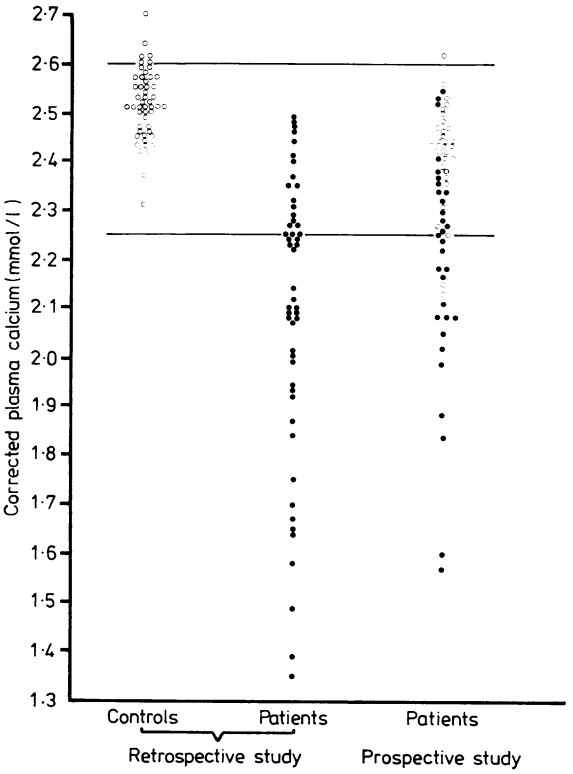

Fig. 1 *Corrected plasma calcium concentrations in the subjects investigated in the retrospective and prospective studies. The open circles indicate subjects with normal bone histology and the closed circles indicate subjects with histological osteomalacia. The normal range for plasma calcium is shown between the two continuous horizontal lines.*

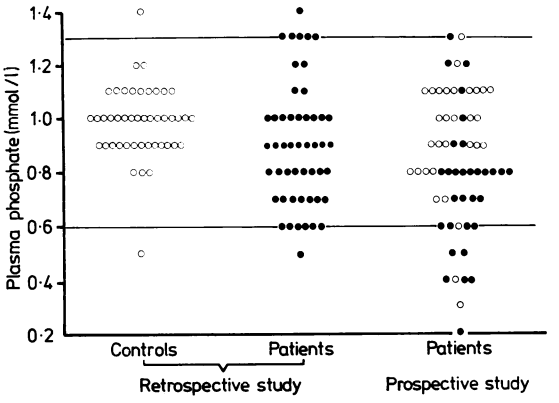

Fig. 2 *Plasma phosphate concentrations in the subjects investigated in the retrospective and prospective studies. Symbols are as in Fig. 1. The normal range is shown between the two continuous horizontal lines.*

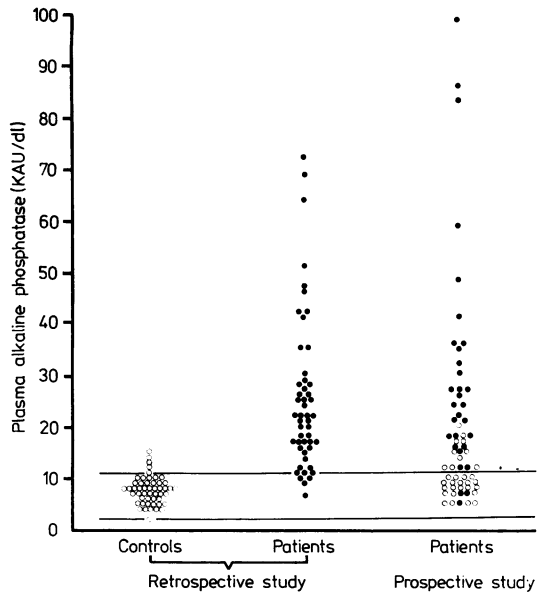

Fig. 3 *Plasma alkaline phosphatase activity in the subjects investigated in the retrospective and prospective studies. Symbols are as in Fig. 1. The normal range is shown between the two continuous horizontal lines.*

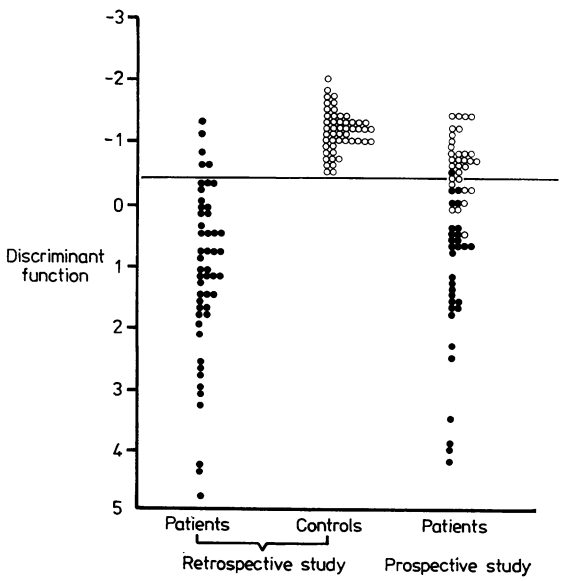

Fig. 4 *Discrimination between osteomalacic and non-osteomalacic subjects in the retrospective and prospective studies using calcium, phosphate and alkaline phosphatase. Symbols are as in Fig. 1.*

Table 3 *False-negative and false-positive rates for histological osteomalacia in retrospective sample*

	False-negative (%)	*False-positive* (%)
Plasma calcium	38	8
Plasma phosphate	96	4
Plasma alkaline phosphatase	14	8
Any biochemical abnormality	10	8
Discriminant analysis of plasma calcium, phosphate and alkaline phosphatase	12	0

calcium concentration and a raised plasma alkaline phosphatase activity, two had only a raised plasma alkaline phosphatase and three had only a raised corrected plasma calcium concentration. The cause of these biochemical abnormalities was not established.

The false-negative and false-positive rates produced by predicting histological osteomalacia from the plasma calcium, phosphate or alkaline phosphatase measurements individually are shown in Table 3. When discriminant analysis was applied to the biochemical data, the lowest false-negative tate (12%) was produced by entering calcium, phosphate and alkaline phosphatase into the analysis together and taking the discriminant score which gave zero false-positives as −0·4 (Fig 4). The formula for calculation of the discriminant score is: 8·51 + (0·4 × alkaline phosphatase) − (3·42 × corrected calcium) − (1·46 × phosphate). The five false-negative results included two of the five patients with normal plasma biochemistry; the remaining three patients had a normal plasma calcium and phosphate concentration and a raised plasma alkaline phosphatase activity (15, 16 and 17 King Armstrong Units/dl; normal range 2-11 KAU/dl). The osteoid volume in these patients ranged from 12·1-28·4% (mean 18·1%).

PROSPECTIVE STUDY (FIGS 1-4, TABLE 4)

Of the 62 patients studied prospectively 31 had histological evidence of osteomalacia, bone histology being normal in the remainder. Three of the patients with osteomalacia had normal plasma biochemistry. Of the 31 patients with normal bone histology, plasma

Table 4 *False-negative and false-positive rates for histological osteomalacia in prospective sample*

	False-negative (%)	*False-positive* (%)
Plasma calcium	48	6·5
Plasma phosphate	81	6·5
Plasma alkaline phosphatase	10	32
Any biochemical abnormality	10	42
Discriminant analysis of plasma calcium, phosphate and alkaline phosphatase	16	10

biochemistry was abnormal in 13; two patients had hypocalcaemia alone, two had hypophosphataemia alone and nine had a raised plasma alkaline phosphatase activity. The use of an abnormal plasma calcium or phosphate concentration again produced high false-negative rates when used to predict histological osteomalacia and, as in the retrospective study, the plasma alkaline phosphatase activity was the most sensitive individual predictor of bone histology. However, the false-positive rate of 32% which followed from plasma alkaline phosphatase measurements was considerably higher than that obtained in the retrospective sample. Similarly, when the presence of an abnormality in any of the three biochemical measurements was used the false-positive rate (42%) was much higher than that found in the retrospective study.

The classification functions derived from the discriminant analysis into which calcium, phosphate and alkaline phosphatase had been entered were used to assign the prospective cases to normal and abnormal bone histology groups. Five cases were incorrectly assigned to the normal group and three were incorrectly assigned to the abnormal group giving a false-negative and false-positive rate of 16% and 9·7% respectively. None of the nine Asians with normal bone histology produced a false-positive result. The three false-positive results comprised two patients with a raised plasma alkaline phosphatase activity (17 KAU/dl in both cases) and one with a low corrected plasma calcium concentration (2·13 mmol/l) and a raised plasma alkaline phosphatase activity (12 KAU/dl). The five false-negative results were obtained in the three patients with osteomalacia who had normal plasma biochemistry and in two patients with a normal plasma calcium and phosphate concentration but a raised plasma alkaline phosphatase activity (15 and 18 KAU/dl). The osteoid volume in these five patients ranged from 10·2-19·0% (mean 13·4).

Discussion

Measurement of plasma calcium, phosphate and alkaline phosphatase is commonly carried out as a diagnostic test for osteomalacia. Our results demonstrate that when an abnormal plasma calcium or phosphate concentration is used to predict histological osteomalacia a high false-negative rate is obtained, although the false-positive rate is low. Plasma alkaline phosphatase activity appears to be the most sensitive single test with which to detect osteomalacia but the low false-positive rate which was found in the case-control study using either an abnormal plasma alkaline phosphatase or any biochemical abnormality could not be replicated in the prospective sample which included patients suspected of having osteomalacia. In contrast, when direct discriminant analysis was applied to the plasma calcium, phosphate and alkaline phosphatase values together the high true positive rate of the retrospective study was replicated in the prospective sample and a false-negative rate of only 16% was obtained. Moreover, the patients in whom false-negative results were obtained had only mild osteomalacia, the mean osteoid volume in these cases being 13·4%.

Since the retrospective study included only non-Asian caucasian controls the inclusion of Asians in this group might have lowered the cut-off point of the discriminant functions, thus increasing the number of false-negatives. However, although biochemical abnormalities suggestive of osteomalacia are relatively common in Asian immigrants, there is no evidence that Asians with normal bone histology differ biochemically from non-Asian caucasians. Moreover, the use of the classification functions based on the retrospective study was validated by the prospective series in which nine Asian patients with normal bone histology were included; of these, none produced a false-positive result.

In this study the discriminant function was based upon biochemical measurements in subjects with normal renal and hepatic function. It is unlikely to be applicable to patients with severe renal dysfunction because of phosphate retention and its secondary effects on the plasma calcium concentration. Similarly, in patients with cholestatic liver disease the increase in the liver isoenzyme of alkaline phosphatase is likely to produce misleading results. In patients without severe renal or hepatic dysfunction however, direct discriminant analysis of the corrected plasma calcium, plasma phosphate and plasma alkaline phosphatase provides a formula which satisfactorily predicts the presence or absence of histological osteomalacia.

Although the use of an abnormal calcium or phosphate concentration on their own both produced high false-negative rates the false-positive rates were low. Calcium would probably have produced a higher false-positive rate if the uncorrected plasma calcium concentration had been used because of the low plasma albumin concentration often associated with malabsorption. The relatively low discriminatory power of alkaline phosphatase is probably due to two factors. First, total plasma alkaline phosphatase is a measure of five different isoenzymes, only one of which originates from bone; raised activities in other isoenzymes, particularly those arising in liver, will cause an increase in the total plasma alkaline phosphatase activity. Separation of the isoenzymes would probably improve the discriminatory

power of alkaline phosphatase, but this procedure is not available in most hospitals. Secondly, whilst causes of hypocalcaemia and hypophosphataemia other than osteomalacia are relatively uncommon, a raised plasma alkaline phosphatase activity, particularly in the elderly, is much less specific for osteomalacia, commonly being due to other causes such as Paget's disease, metastatic bone disease or obstructive liver disease.

Calculation of the discriminant score from plasma calcium, phosphate and alkaline phosphatase using the formula we have described will probably be useful when these measurements are used to screen populations, since false-positive and false-negative rates, once correctly identified by combined biochemical and histological studies, do not reduce the usefulness of a diagnostic test used for this purpose. However, in the assessment of individual patients biopsy remains the only certain method of the diagnosis of osteomalacia in the absence of unequivocal clinical, biochemical or radiological signs.

Since false-negative rates are less significant in subpopulations which have a high prevalence of a disease, the discriminant function we have devised should be particularly useful in determining the prevalence of osteomalacia in the Asian immigrant population and in the elderly British population. In the Asian immigrant population clinical, biochemical and radiological studies have estimated that from 5 to 30% of children and adult women have overt rickets or osteomalacia, whilst up to 74% of children and 53% of adults have biochemical abnormalities suggestive of rickets or osteomalacia.[5 6] Low plasma 25-hydroxyvitamin D concentrations have been reported in 78% of a group of Asian schoolboys,[7] over 50% of Hindu adult women[8] and 81% of pregnant Asian women.[9] These figures almost certainly overestimate the prevalence of histological osteomalacia, but nevertheless indicate the high risk of rickets and osteomalacia in this population. The prevalence of vitamin D deficiency and osteomalacia is also believed to be increased in the elderly British population[10–12] particularly those living in institutions.[13] The discriminant function enables plasma estimates of calcium, phosphate and alkaline phosphatase to be used not only in determining the prevalence of histological osteomalacia in these populations but also to assess the effectiveness of public health measures such as vitamin D supplementation or fortification of food with vitamin D.

We thank J Sainsbury Ltd and the Special Trustees, St Thomas' Hospital for generous financial support. We are grateful to Adrian Webb for preparation of the bone histological sections.

References

1 Chalmers J, Conacher WDH, Gardner DL, Scott PJ. Osteomalacia—a common disease in elderly women. *J Bone Joint Surg* [*Br*] 1976;**49**:403-23.

2 Compston JE, Horton LWL, Laker MF, *et al.* Bone disease after jejuno-ileal bypass for obesity. *Lancet* 1978;ii:1-4.

3 Payne RB, Little AJ, Williams RB, Milner JR. Interpretation of serum calcium in patients with abnormal serum proteins. *Br Med J* 1973;iv:643-6.

4 Nie NH, Hull CH, Jenkins JG, Steinbrenner K, Bent DH. *Statistical package for the social sciences.* 2nd ed. London and New York: McGraw-Hill Book Company, 1975;434-67.

5 Goel KM, Sweet EM, Logan RW, Warren JM, Arneil GC, Shanks RA. Florid and subclinical rickets among immigrant children in Glasgow. *Lancet* 1976;i:1141-8.

6 Holmes AM, Enoch BA, Taylor JL, Jones ME. Occult rickets and osteomalacia amongst the Asian immigrant population. *Q J Med* 1973;**42**:125-49.

7 Ellis G, Woodhead JS, Cooke WT. Serum 25-hydroxyvitamin D concentrations in adolescent boys. *Lancet* 1977;i:825-8.

8 Hunt SP, O'Riordan JLH, Windo J, Trusswell AS Vitamin D status in different subgroups of British Asians. *Br Med J* 1976;ii:1351-4.

9 Heckmatt JZ, Peacock M, Davies AEJ, McMurray J, Isherwood DM. Plasma 25-hydroxyvitamin D in pregnant Asian women and their babies. *Lancet* 1979; ii:546-8.

10 Anderson I, Campbell AER, Dunn A, Runciman JBM. Osteomalacia in elderly women. *Scott Med J* 1966;**11**: 429-35.

11 Jenkins DHR, Roberts JG, Webster D, Williams EO. Osteomalacia in elderly patients with fracture of the femoral neck. A clinico-pathological study. *J Bone Joint Surg* [*Br*] 1973;**55**:575-80.

12 Exton-Smith AN, Hodkinson HM, Stanton BR. Nutrition and metabolic bone disease in old age. *Lancet* 1966; ii:999-1001.

13 Vir SC, Love AHG. Nutritional status of institutionalised and non-institutionalised aged in Belfast, Northern Ireland. *Am J Clin Nutr* 1979;**32**:1934-47.

Requests for reprints to: Dr Juliet Compston, Gastrointestinal Research Unit, Rayne Institute, St. Thomas' Hospital, London SE1 7EH, England