5

Nutritional Osteomalacia and Late Rickets in Greater London, 1974—1979: Clinical and Metabolic Studies in 45 Patients

T. C. B. STAMP
P. G. WALKER
W. PERRY
M. V. JENKINS

CLINICAL AND EPIDEMIOLOGICAL FEATURES

The study of nutritional osteomalacia has among its main landmarks the histopathological studies in Germany by Pommer (1885; see Aaron, 1978), the clinical and epidemiological surveys by Palm (1890), the clinical and radiological descriptions in Germany by Looser (1920a,b) and the classic series of biochemical studies and therapeutic experiments with vitamin D in Northern China in the 1930s (Hannon et al, 1934; Liu et al, 1935; Maxwell et al, 1939; Liu and Chu, 1943). With its easy means of prevention, clinical disease from vitamin D deprivation became rare in Europe and the western hemisphere and a generation ago Albright and Reifenstein (1948) were 'cognizant of no single case of osteomalacia in the United States due to simple vitamin D lack. . .'.

Reports then began to accumulate of apparent privational disease among patients who had had stomach operations, especially partial gastrectomy — up to many years previously, and several groups in Australia (Deller and Begley, 1963; Deller, Edwards and Addison, 1963), the United States (Eddy, 1971) and England (Morgan, Hunt and Paterson, 1970) surveyed the clinical, radiographic, histological and biochemical features of this form of complicated deficiency disease. Patients tended to be in the older age-groups, only 11 out of 23 surveyed by Morgan, Hunt and Paterson (1970) being below the age of 60. Occasional cases of pure deficiency were also recognized among the elderly, particularly in females (Gough, Lloyd and Wills, 1964; Berthaux, Laurent and Beck, 1970; Rosin, 1970). A survey of elderly females in London suggested that osteomalacia contributed

significantly to a reduction that was found in their bone density (Exton-Smith, Hodkinson and Stanton, 1966): proper perspective of the incidence of *disease* needed to be maintained by noting that none had any relevant symptoms. Comprehensive surveys have amalgamated the gastric-surgery and elderly groups and included patients with malabsorption syndromes (Chalmers et al, 1967; Morgan, 1973). Together they provided detailed information on the scale of clinical symptoms (bone pain, tenderness and muscle weakness), radiological disturbance (number and distribution of Looser zones, and phalangeal subperiosteal erosions from secondary hyperparathyroidism) and of biochemical abnormalities (normal or low plasma calcium and phosphorus and variable hyperphosphatasia) in these particular groups. Dent and Smith (1969) described seven patients with nutritional osteomalacia of whom three were young (aged 20 to 46) white females and the authors delineated this new group of younger 'food-faddists' who avoided all vitamin D-containing fatty foods.

Pure privational osteomalacia is still endemic in the developing world, with accounts from India (Vaishnava and Rizvi, 1967), Iran (Chapman, 1971) and even in Bedouin females in the Negev desert who never leave their goatskin tents (Groen et al, 1965; Paterson, 1973). In Britain, war-time statutory fortification of food with vitamin D was discontinued in the early 1950s. Although vitamin drops are available under the Government Welfare Foods Scheme, the unforeseen scale of Asian immigration reintroduced the clinical problem of rickets in this country. Thus, the disease became recognized in this Asian population among infants and children (Dunnigan et al, 1962; Arneil and Crosbie, 1963; Benson et al, 1963; Ford et al, 1972), pregnant mothers (Felton and Stone, 1966) and in other adults (Dent and Smith, 1969). It remained very rare among black immigrants, however, casting considerable doubt on the popular view that white skin is advantageous to vitamin D nutrition (Loomis, 1970; Bronowski, 1973; Stamp, 1975).

An important epidemiological survey of 168 randomized Asian immigrants, mainly from Kashmir and the Punjab, revealed what the authors interpreted as clinical evidence of otherwise 'occult' rickets or osteomalacia in 30 per cent and biochemical abnormalities in 74 per cent (Holmes et al, 1973). It is noteworthy that no subject admitted to symptoms of their presumed deficiency nor did any adult show radiographic evidence of osteomalacia. On the basis of the high prevalence and the distribution of osteomalacia in their subjects' country of origin, the authors concluded that residence in Britain per se did not contribute to the development of disease in that population. The presence or absence of morbidity in the study was, and is, likely to influence public health decisions with implications for the future. Both these important points are discussed further in the following section.

It was apparent from the studies listed above that a detailed survey of overt uncomplicated nutritional late rickets and osteomalacia had not been reported previously. The present study fills this gap. It also provided the opportunity to compare the relative efficacy of the metabolites 25-hydroxycholecalciferol ($25OHD_3$), 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$), 24R,25-dihydroxycholecalciferol ($24,25(OH)_2D_3$) and 1α-hydroxycholecalcif-

erol (1α-OHD_3). Finally, follow-up during long-term treatment verified certain patterns of vitamin D activity, particularly in relation to phosphorus retention, that had not been established previously in the literature.

The present study was based on 45 patients referred to us at the Royal National Orthopaedic Hospital between 1974 and 1979, in whom the diagnosis of privational disease was made by exclusion.

Overt Nutritional Osteomalacia (and Late Rickets) in Greater London

All but one of 45 patients presented with symptoms directly attributable to rickets or osteomalacia (see below), the exception being an 88-year-old white woman with a history of 'repeated falls'. All patients with symptoms, signs, clinical or biochemical evidence that ultimately showed gastrointestinal or renal disease, including those of our patients who had had partial gastrectomy, were excluded from the present series.

Symptoms consisted of bone pains, muscle weakness and tetany. Out of the total population 87 per cent complained of mild to incapacitating bone pain: pain was situated in the back in 50 per cent, below the hips (knees, ankles, feet) in 33 per cent, in the hips in 25 per cent and in ribs in 25 per cent; rarer sites were shoulders and arms and most patients actually complained of their weakness. Two young patients complained only of their inability to run. Tetany was uncommon, only three patients (7 per cent) admitting to carpo-pedal spasm and a fourth admitting to 'tingles'. Two adolescents with the least symptoms had genu varum with 9.5 and 12.5 cm separation between medial malleoli.

All but two elderly female patients were of Asian origin. Thirty-five came from East Africa (Kenya, Tanzania and Uganda), two further Asian adolescents were born in Britain, and six patients came from the Indian subcontinent. Their age and sex distribution is shown in Figure 1. Length of residence in Britain was recorded in 33 Asian patients: none had been in Britain for less than three years and life-long residence was recorded in two sibs aged 12 and 15. Duration of symptoms, recorded in 32, ranged from two months to five years with a median duration of >10 <12 months. No Asian patient had developed symptoms before they had been in Britain for at least two years. In 27 patients whose symptoms had not lasted more than 12 months their duration was traced back to the month of onset (Figure 2), represented in two-month periods and revealing a tendency for symptoms to develop between mid-winter and late spring. This was fully consistent with the seasonal nadir of vitamin D nutrition that occurs at the same time in white subjects (Haddad and Stamp, 1974; McLaughlin et al, 1974; Stamp and Round, 1974) and in Asian immigrants (Gupta, Round and Stamp, 1974). Three patients volunteered improvement in their symptoms during summertime. Other diseases included past and present treatment for tuberculosis (seven), mental deficiency (one), epilepsy and anticonvulsant therapy (one), previous 'glutethimide addiction' (one), diabetes mellitis (one), rheumatoid arthritis on corticosteroid therapy (one), and old poliomyelitis and mid-thigh amputation (one). Attempts to incriminate antituberculous therapy with the enzyme-inducing drug rifampicin were unsuccessful: one patient who

developed symptoms while receiving rifampicin went back to Kashmir with both diseases healed and presented immediately on return, one year later, with osteomalacia again. Two other patients receiving rifampicin developed strong positive calcium balances with very small doses of vitamin D, excluding a metabolic (i.e. 'vitamin D-resistant') aetiology for their disease. Despite the arguments of Holmes et al (1973, see above) it is also possible that residence in India may provide *better* vitamin D nutrition than residence in England. Figure 3 shows 'spontaneous' healing of gross rickets in an 'untreated' 14-year-old Indian boy over a three-month period during which he returned to India (Gujerat) for a six-week holiday.

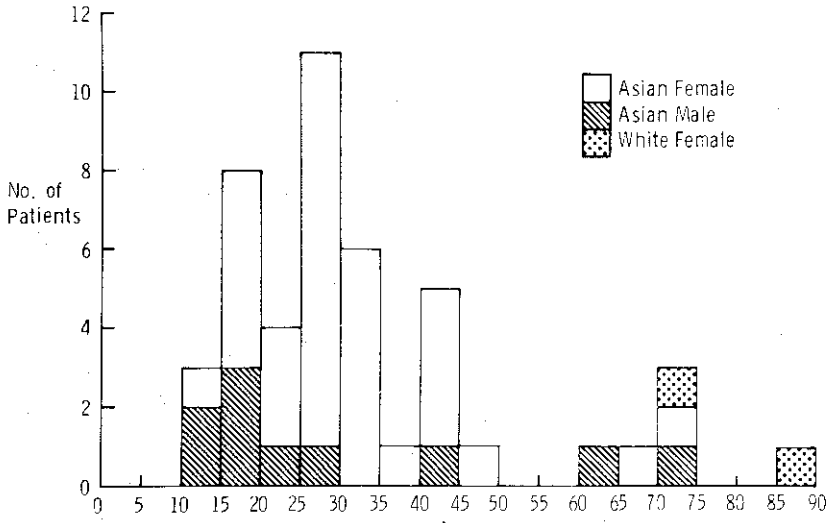

Figure 1. Age, sex and racial origin in 45 patients with nutritional late rickets and osteomalacia.

Physical signs of bone tenderness were elicited by spinal percussion, sternal compression and lateral rib compression. While it was not always confirmed in retrospect that these signs were *sought* in each patient, *positive* signs were recorded in the spine in 50 per cent, over the lateral ribs in 42 per cent and over the sternum in 38 per cent. Objective evidence for myopathy was more difficult to record. Difficulty in rising from a low chair or from the squatting position was noted in 36 per cent; abnormal gait, usually referred to as 'waddling', was, and is, a very subjective observation (particularly in young females); for what it is worth it was recorded in 47 per cent.

The presence or absence of Chvostek's and Trousseau's signs, determined in the standard manner (Smith, 1979) was noted in 35 patients: 12 out of the 35 had a positive Chvostek's sign (defined either as more than a flicker of the levator labii superioris or response from more than one facial muscle). Only

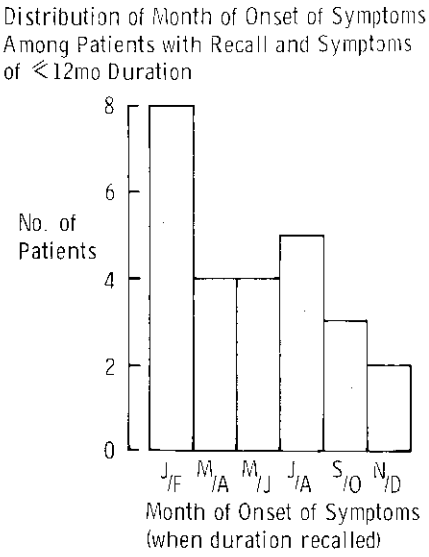

Figure 2. Seasonal onset of symptoms, when not longer than 12 months' duration, in 27 patients whose recall permitted onset to be dated to a given month.

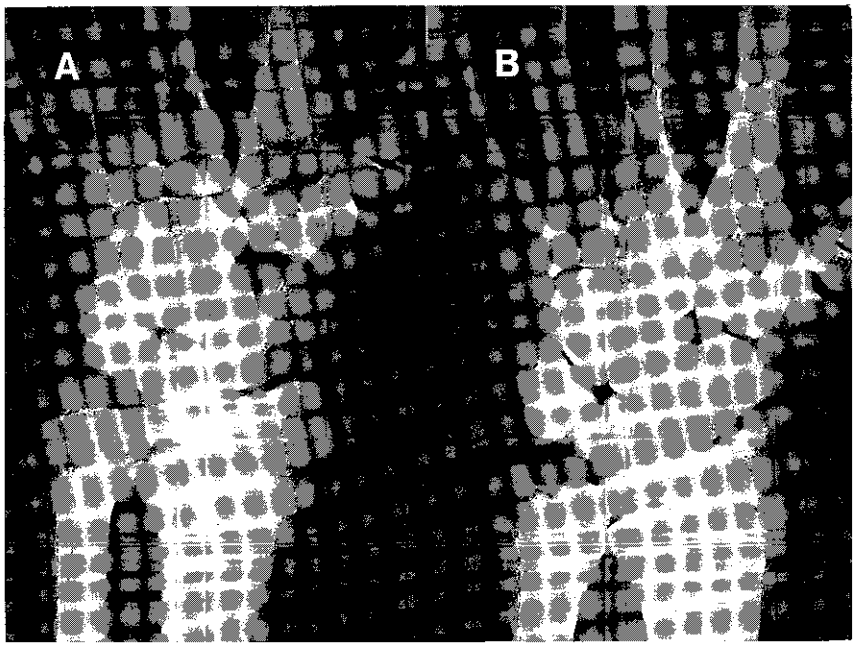

Figure 3. 'Spontaneous' cure of radiological rickets between the months of (end-) June and October in a 14-year-old Asian boy, associated with a six-week holiday in Mid-India (Gujerat) during this period. A. 22 June. B. 14 October.

five out of 33 patients had a positive Trousseau's sign. It was of interest that none of the three patients who complained of carpo-pedal spasm had a positive Chvostek's sign and in one of them Trousseau's sign was also negative.

Electromyography was performed in eight patients with proximal weakness. Polyphasic action potentials indicative of (non-specific) myopathy were present in four but negative results were common and in one normal and abnormal areas were seen side by side in the same quadriceps muscle. We conclude that electromyography is unhelpful in the investigation of osteomalacic myopathy.

Full skeletal radiology was performed in every patient. All adolescents showed prominent rickets; widening or fuzziness of the iliac and ischial apophyses was a helpful sign up to the age of 19. Either rickets or Looser zones were present in all but 10 patients. Twenty-six patients had 53 Looser zones (an average of just over two each), 17 in femoral neck, three in upper femoral shaft, 17 in superior or inferior pubic rami, 11 in ribs, two in the lateral scapula, two in the ulna and one in the fibula. This distribution of Looser zones is similar to that reported by Chalmers et al (1967). Only one radius and ulna, and one tibia and fibula were x-rayed in each patient so, even assuming twice the present incidence of long-bone abnormalities, close on 90 per cent of all 'pseudofractures' are revealed by two radiographs, chest and pelvis. Some of these abnormalities were regarded initially only as 'suspicious', later being confirmed by clear changes following vitamin D treatment. Subperiosteal erosions in phalanges indicative of secondary hyperparathyroidism were found in 15 of the 45 patients (33 per cent). These changes were, for uncertain reasons, best seen along the radial border of the middle phalanx of the index finger.

Quantitative histology was derived from iliac crest biopsy in 17 patients after in vivo tetracycline labelling. All showed gross changes with osteoid coverage greater than 50 per cent (normal, less than 27 per cent). Complete histological data, in relation to abnormal biochemistry, radiological hyperparathyroidism, etc. are in preparation for later publication.

Discussion

Two features of the disease as it currently presents in Greater London are worthy of comment. First, the number of patients suffering symptoms of many months' duration presenting to one hospital in an area with a relatively low immigrant population indicates widespread morbidity that fortification of food with vitamin D could prevent. Moreover, the problem is not diminishing; nine of the 45 patients in the present series presented during the first eight months of 1979. Nor is the end of the problem in sight; the young females in our series had rigid vegetarian dietary habits (see 'Biochemistry and Vitamin D Nutrition'), which they will undoubtedly foster in their offspring. The other question is the effect of residence in Britain. Holmes et al (1973) argued persuasively against such an effect in their asymptomatic patients from the Indian subcontinent. Their contention was based on the frequency of disease in India; they quoted the findings of Vaishnava and

Rizvi (1971) that 98 per cent of patients in New Delhi were females aged between 15 and 30. Our study suggests that the situation in Asian immigrants from East Africa (Kenya, Tanzania and Uganda) may be different. The incidence of osteomalacia in East Africa is even less certain than it is in Pakistan, Kashmir and Northern India. Nevertheless it is striking that none of our 33 patients from Africa had developed symptoms before they had been at least two years in Britain. Many females were over the 30-year 'age limit' that Vaishnava (1975) found in Delhi, suggesting that the age distribution differs between England and India. More importantly, plasma 25-hydroxyvitamin D levels measured in immigrants on arrival from Uganda in November 1972 showed no evidence of vitamin D-deficiency (see page 91). We believe that however similar the prevalence of osteomalacia may (or may not) be between Asian immigrants in Rochdale, Lancashire (latitude 53°N) and natives of the Punjab (latitude 30-32°N), emigration to Britain from equatorial Uganda (latitude 0°) may precipitate *clinical* osteomalacia in vegetarian subjects.

BIOCHEMISTRY AND VITAMIN D NUTRITION IN 'CLASSICAL' OSTEOMALACIA (AND LATE RICKETS)

The variability of hypocalcaemia and/or hypophosphataemia in rickets and osteomalacia has been known since the original studies of Howland and Kramer (1921,1922) who also recorded occasional hyperphosphataemia among children and adolescents. This latter biochemical appearance of 'functional hypoparathyroidism' has been discussed in detail (Arnstein, Frame and Frost, 1967). The pattern of low urinary and high faecal calcium excretion was established by early metabolic studies (Liu et al, 1935; Liu and Chu, 1943) and Dent and Smith (1969) showed that urinary calcium excretion may remain low for months during the healing phase. Variable elevations of plasma alkaline phosphatase activity and, to a lesser extent, urinary total hydroxyproline excretion have been surveyed by Morgan (1973). Other biochemical features of osteomalacia have been reported including reduction in plasma total bicarbonate, attributed to decreased renal tubular bicarbonate reabsorption (Muldowney, Freaney and McGeeney, 1968; Vainsel, Manderlier and Vis, 1974), and variable aminoaciduria (Fraser, Kooh and Scriver, 1967; Vainsel et al, 1974). Both these features resolve during treatment and are thought to result from secondary hyperparathyroidism.

Measurement of circulating 25-hydroxyvitamin D (25OHD) has become accepted as a good index of vitamin D nutrition although its relationships to the level of cutaneous vitamin D synthesis and to body tissue stores under physiological conditions is uncertain. Low levels were first reported in Asian immigrants by Preece et al (1973) although these authors neglected the importance of seasonal variation in circulating 25OHD. This parameter was studied in a group of Asians by Gupta, Round and Stamp (1974). Half of them had hypocalcaemia in the spring which was associated with 25OHD levels below 5 ng/ml; in all but one normocalcaemia was 'spontaneously' restored by the autumn and the mean 25OHD levels had risen from 9.9 to

14.7 ng/ml ($P<0.01$ for paired values). The distribution of circulating 25OHD concentrations is skewed upwards in the population and the corresponding log-transformed means were, after re-conversion, 6.9 and 12.1 ng/ml respectively. Circulating 25OHD in healthy white subjects in London varied from a minimum of 12.9 (range 6.6-25.2) ng/ml in March/April to a maximum of 22.6 (range 13.4-37.9)ng/ml in September/October. Plasma 25OHD of 7.9 ng/ml is therefore quite compatible with clinical, radiological and biochemical rickets presenting in the month of September, as has been reported (Dent et al, 1973). We examined these and other laboratory indices in our patients before and during treatment with the vitamins D. We further report a preliminary survey of plasma 25OHD among Ugandan Asian immigrants that was undertaken on their arrival in Britain during November 1972.

Methods of Study

Standard laboratory methods were used. Calcium and magnesium were measured by atomic absorption spectrophotometry and the former was corrected for serum-specific gravity, measured by refractometry to four decimal places; protein electrophoresis confirmed normal or near-normal protein distribution in all: a calcium value of 0.06 mmol/l is added or subtracted for each change in the third decimal place below or above 1.0270 respectively; smaller changes are calculated from the fourth decimal reading (Berry et al, 1973). Serum alkaline phosphatase was measured by an automated technique (Axelsson, Eckman and Knutson, 1965), and 25OHD by the method of Haddad and Chyu (1971), a chromatographic step being regarded as essential. Urine amino acid excretion was measured semi-quantitatively by two-dimensional paper chromatography after adjusting dilution to constant creatinine concentration (Smith, Seakins and Dayman, 1969).

Results and Discussion

Plasma urea and creatinine were not raised in any patient (particularly low levels of urea were a feature of vegetarian diets), routine urinalysis was likewise normal (except for glycosuria in one patient with diabetes) and significant proteinuria was absent. Abnormalities of faecal fat excretion, oral glucose tolerance and barium follow-through radiography were excluded in all patients in whom intestinal malabsorption could be suspected (e.g. any history of dyspepsia, diarrhoea or if osteomalacia was particularly severe), faecal fat excretion being measured during all metabolic balance studies.

Mean serum calcium was 2.17 ± 0.15(s.d.)mmol/l, pre-treatment values being normal (>2.20 mmol/l) in almost exactly half (22) of our patients. Distribution of serum calcium values showed a downward skew (Figure 4), hypocalcaemia being most severe among the adolescent population with late rickets (range 1.67-1.95 mmol/l). Serum magnesium measured in eight patients was in the mid-normal range, mean 0.91 ± 0.08(s.d.)mmol/l, range 0.78-1.09. Mean serum alkaline phosphatase on log-transformed values was 22.6 K-A units (statistical range 6.3-81) and its skewed distribution is shown

in Figure 4. Dent and Harper (1962) reported a sex difference in normal adult phosphatase levels with a strict upper normal limit of 10 KAU in females and 12 KAU in males. Three Asian females in the present series thus had unequivocally normal serum alkaline phosphatase (10 KAU in two, 7 KAU in one — each the mean of three pre-treatment samples). *All had presented with marked symptoms* (bone pain). One was both normocalcaemic and normophosphataemic yet her x-rays showed two Looser zones and subperiosteal erosions and bone biopsy showed excess osteoid,

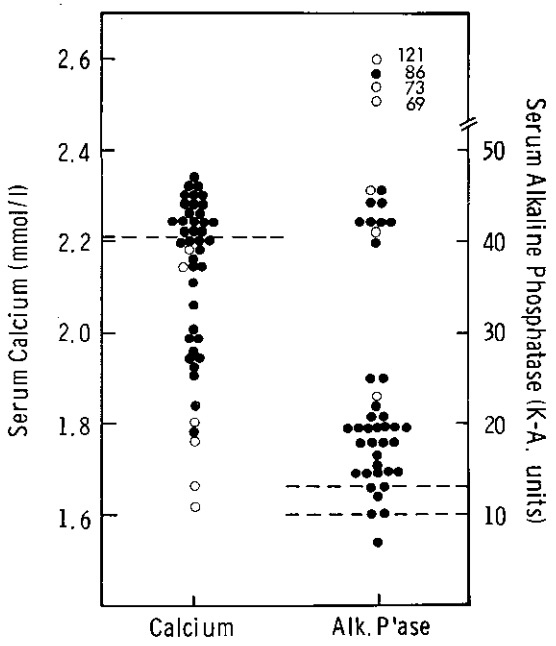

Figure 4. Distribution of serum calcium and alkaline phosphatase levels among 45 patients with nutritional late rickets (○) and osteomalacia (●). Note that normocalcaemia occurred in 50 per cent and serum alkaline phosphatase was unequivocally normal in three patients. Broken lines indicate lower limit of normal for calcium and upper limits of normal serum alkaline phosphatase for males and females (see text).

'obvious' resorption and perilacunar mineral loss; serum phosphatase rose to a maximum of 12 KAU during treatment. A second was hypocalcaemic but normophosphataemic and her x-rays were likewise normal; bone biopsy showed greater than 50 per cent osteoid coverage and widespread perilacunar mineral deficiency; phosphatase rose to frankly high level during treatment. The third (serum phosphatase 7 KAU) was normocalcaemic but mildly hypophosphataemic; x-rays showed a Looser zone and subperiosteal erosions (bone biopsy not performed); phosphatase did not rise during treatment.

These findings confirm that clinical, radiological and histological osteomalacia may occur without abnormally high serum phosphatase (see also long-term results). Plasma phosphorus normally varies both with age and sex. Seventeen of the 38 adults in the present series were hypophosphataemic (serum P<0.8 mmol/l) with a mean of 0.84 ± 0.18(s.d.)mmol/l. Among children with rickets two biochemical 'sub-groups' have long been recognized (Howland and Kramer, 1922), those with low calcium and high phosphorus and those with normal or near-normal calcium and low phosphorus. The existence of similar sub-groups among adults has been debated but the balance of evidence, based on exhaustive probability analysis (Morgan, 1973) is against this distinction. There was certainly no suggestion of such sub-grouping among our patients. Morgan (1973) also commented on the (expected) association between low plasma phosphorus and radiological hyperparathyroidism. While such a tendency was apparent among our adult patients it was by no means universal; subperiosteal erosions were seen with a serum phosphorus level of 1.26 mmol/l and were absent in two patients with serum phosphorus of 0.46 and 0.55 mmol/l.

Mean plasma bicarbonate was 22.3 ± 2.3(s.d.)mmol/l (normal range 20-30). While this was not significantly different from normal, five patients had bicarbonate levels of 17-19 mmol/l. Plasma bicarbonate was recorded after treatment in eight patients, who showed a mean rise of 1.6 ± 3.5(s.d.)mmol/l. This change was not significant and more complete follow-up is in progress.

Relative aminoaciduria was reported in 11 of 38 patients, confirming many previous reports (Fraser, Kooh and Scriver, 1967; Muldowney, Freaney and McGeeney, 1968; Vainsel et al, 1974). Possible changes following treatment were not recorded.

Dietary survey suggested that both vitamin C and iron intakes were low in some patients. Vitamin C saturation tests in three patients confirmed relative deficiency but no symptoms or signs of vitamin C deficiency were apparent.

Ten of 45 patients showed evidence of iron deficiency with haemoglobin levels below 12 g/100ml (mean Hb in *these* subjects was 10.5 ± 1.3(s.d.)g/dl. All responded to oral iron and no macrocytic component emerged. Folate and B_{12} levels were normal when measured.

Serum 25OHD was profoundly low in nearly all the 32 subjects in which it was measured with a mean of less than 3.5 ng/ml; thus, while 26 subjects had a mean of 3.5 ng/ml (range 0.8-5.9 ng/ml with an outside value of 8.9 ng/ml) undetectable levels were found in six further subjects; four in assays in which serum dilutions were such that the limit of detection was ⩽2.2 ng/ml and two in which the limit was 4.1 ng/ml.

Dietary histories were not easy to establish in the immigrant group since many items in their diet are not listed in tables of vitamin D content compiled for Western diets. As far as could be told all had a 'very low' vitamin D intake (i.e. 20-30 iu daily at most). Although deficient ultraviolet exposure was easy to presume in most patients it was not always apparent; several, including males, walked part of their way to work and one adolescent played cricket once a week. While classical theories of dietary and ultraviolet deprivation as the sole cause(s) of classical osteomalacia rightly prevail they perhaps cannot yet be regarded as wholly watertight.

Vitamin D nutrition in New East African Asian Immigrants

The question whether residence in England may precipitate rickets and osteomalacia in vegetarian subjects of Asian origin could be clarified by surveying plasma 25OHD in Asians within their country of origin. Rickets and osteomalacia are uncommon in Hyderabad, India (latitude 17°N) and plasma 25OHD in healthy subjects frequently ranges between 20-35 ng/ml (Vinodini Reddy, personal communication). While 25OHD levels among Asians in East Africa have not been reported a survey was undertaken among new arrivals from Uganda during a period of rapid immigration in November, 1972 (Stamp, 1980). Plasma was sampled from Asian volunteers within a transit camp at Stradishall, Essex.

A mean 25OHD in 13 Ugandan children (average age 11 years) of 17.1 ± 6.8 ng/ml was significantly lower than the mean in 13 English children (average age 11) of 26.5 ± 4.8 ng/ml measured in a single assay ($P<0.01$). The English children had been sampled during September and early October. Ten Ugandan Asian females aged 15 to 20 years had a mean plasma 25OHD of 16.2 (range 9-28)ng/ml while in the same assay 10 normal English females aged 16 to 17 years who were sampled in January had a mean 25OHD of 18.6(range 13-39)ng/ml, and 10 healthy elderly subjects (aged over 70 years) had a mean of 17.5 (range 8-38)ng/ml. None of these last comparisons differed significantly. Results in the full English sample have been reported previously (Stamp and Round, 1974). Hypocalcaemia was not observed in any of a full Asian sample of 263 subjects.

These data strongly suggest that although Asians in Uganda may have had lower 25OHD levels than the healthy English seasonal maximum they did not come to this country suffering from vitamin D deficiency.

TREATMENT OF RICKETS AND OSTEOMALACIA WITH THE VITAMINS D: METABOLIC BALANCE STUDIES

The human physiological requirement for $1,25(OH)_2D$ with respect to oral administration of the hormone has been difficult to establish. Balsan et al (1975) reported good healing of nutritional rickets with doses of 50 ng/kg/day; Chesney et al (1978) found improved growth in childhood renal osteodystrophy with the minimum doses of 14 ng/kg/day that they used. In vitamin D-dependency rickets, a disease characterized by metabolic block in the formation of $1,25(OH)_2D$, Fraser, Kooh and Scriver (1978) obtained complete eventual biochemical and radiological healing in an infant treated with 'less than 10 ng/kg/day'. Studies in normal man and in patients with renal failure led other workers to suggest that the amount of $1,25(OH)_2D_3$ being turned over normally may be less than 100-700 ng/day (Brickman, Coburn and Massry, 1974).

Previous metabolic balance data suggested that privational late rickets and osteomalacia healed slowly if dosage of $1,25(OH)_2D_3$ was less than 1 μg/day: higher doses produced rapid biochemical response, strong mineral retention and complete radiological healing (Stamp et al, 1979). $24,25(OH)_2D_3$ alone

was without significant effect. This evidence conflicts with work suggesting that other metabolites may indeed exert physiological effects. $24,25(OH)_2D_3$ promoted bone mineralization in chicks (Ornoy et al, 1978) and was important in the whole (egg-laying) life cycle of this species (Norman and Henry, 1979). Some enhancement of radiocalcium absorption by $24,25(OH)_2D_3$ was reported in human renal osteodystrophy and appeared more pronounced in other conditions (Kanis et al, 1979). Furthermore, clinical and histological studies suggested that, while $1,25(OH)_2D_3$ alone increased intestinal calcium absorption and reduced secondary hyperparathyroidism it did not enhance renal tubular phosphate reabsorption. $1,25(OH)_2D_3$ did not restore normal mineralization front by comparison with treatment using either $25OHD_3$ or a combination of $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$. Combined treatment using $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ did not, however, raise plasma phosphorus or TmP/GFR by comparison with $25OHD_3$ itself (Bordier et al, 1978). In order further to estimate the physiological requirement for $1,25(OH)_2D_3$ and to examine a possible potentiation of its healing effect by $24,25(OH)_2D_3$, we extended our original study (Stamp et al, 1979) to metabolic balance investigation in six additional patients with nutritional rickets or osteomalacia.

Methods of Study

Balance studies were performed in the classical manner (Albright and Reifenstein, 1948) as modified by Dent and Smith (1969). Successive four-day faecal collections were separated by carmine markers. An 'internal' marker of cuprous thiocyanate was given orally, divided among meals in a total daily dose of 250 mg (Dick, 1969). Faecal calcium and phosphorus contents were then 'corrected' according to faecal recovery of copper. The salt is safe and copper analysis by atomic absorption spectrophotometry is simple and accurate. Urines were analysed in 48-hour collection periods and balances were expressed as a mean daily figure. Construction of palatable Asian diets created difficulties that were overcome by bulk cooking of curries jointly by the dietitian and the patients (or the female cook in their household) themselves. Cooked stocks for the whole of the planned balance study were frozen. Duplicate diets were analysed whenever separate batch cooking was required. As in previous studies stability of $24R,25(OH)_2D_3$ was confirmed by high pressure liquid chromatography (courtesy T. L. Clemens and Dr J. L. H. O'Riordan) when each batch was nearly exhausted.

Results and Discussion

Recalculation of our previous results (Stamp et al, 1979) showed that, on body-weight-related dosage, $1,25(OH)_2D_3$, 17-34 ng/kg/day produced calcium retention *at least* equal to 40 per cent of intake while two patients who had received 7 and 12 ng/kg/day showed only 12 and 15 per cent calcium retention respectively; these last figures represented absolute values of 3.8 and 4.1 mmol calcium daily and barely indicated significant response.

Table 1 shows the relationship between calcium intake, per cent retention and dosage of the vitamins D in six Asian females aged 12 to 35 years. $1,25(OH)_2D_3$ was given alone at first; $24,25(OH)_2D_3$ was subsequently added in four patients, and $25(OH)D_3$ was finally substituted in three patients. Dietary mineral was supplemented in patients 3 to 6 by microcrystalline hydroxyapatite compound (M.C.H.C., Ossopan) in order to study response to high calcium intake in as 'physiological' a form as possible. Retention was recorded as the mean of control periods (when measured) or as the maximum that was observed over any one of two or three consecutive four-day treatment periods.

Patients 1 and 2 showed only small changes in calcium retention on low doses of $1,25(OH)_2D_3$. Patient 2, with very severe rickets, was unimproved clinically, biochemically or radiologically after two months' treatment despite added $24,25(OH)_2D_3$ 10 μg daily for eight days and a subsequent bolus of $24,25(OH)_2D_3$ 200 μg at the end of her balance study. Moreover maximal positive balance of 7.0 mmol/day was no more than the normal retention expected during pubertal growth. The response in patient 3 was clearer although it was still relatively low (Dent and Smith, 1969; Stamp et al, 1979). Although further studies (in progress) are clearly desirable we conclude on present evidence that the *oral* requirement for $1,25(OH)_2D$ in humans, when replacing vitamin D from skin and from diet, may be close to 7 ng/kg/day.

A barely-detectable improvement in calcium retention appeared to follow addition of $24,25(OH)_2D_3$ in the three patients who received it. Minor simultaneous changes in phosphorus balance were appropriate and we therefore believe our observations to be genuine. In two of the three patients the trivial improvement in phosphorus balance (1.8 and 3.0 mmol daily) was due to a fall in faecal phosphorus and not to a fall in urinary phosphorus which would *otherwise* suggest increasing renal tubular phosphate reabsorption. The third patient improved by only 1.3 mmol daily and the change could not be apportioned. These changes could have occurred as a secondary response to changes in calcium rather than to any primary effect of vitamin D. Whatever the significance of this response to $24,25(OH)_2D_3$ it is plainly of negligible importance to mineral retention *in osteomalacia.*

$25OHD_3$ (40 μg/day) was substituted in three patients (numbers 3 to 5), enhancing mineral retention in two of them, patient 3 on low-dose $1,25(OH)_2D_3$ with added $24,25(OH)_2D_3$, and patient 5 on high-dose $1,25(OH)_2D_3$. Patient 4 had been on high-dose $1,25(OH)_2D_3$ for two months and her mineral retention (conversely) declined simultaneously with substitution of $25(OH)D_3$. Patient 6 on the highest dose of $1,25(OH)_2D_3$ (4 μh/day) showed massive calcium retention of 35.6 mmol daily while urine calcium never rose above 1.0 mmol daily. Similar healing rates may be produced also by vitamin D_3 250 μg daily, $25OHD_3$ 40 μg daily and by ultra-violet light (Stamp et al, 1979); in particular the efficacy of $25OHD_3$ 40 μg/daily, now reported from metabolic balance studies in seven patients, has been equivalent to that of $1,25(OH)_2D_3$ in doses of 1-4 μg daily. This effective dose-ratio contrasts strikingly with the ratio of their concentrations in plasma, the level of $1,25(OH)_2D_3$ rarely being more than 1/400 that of

Table 1. *Metabolic balance data in 6 Asian females aged 12 to 35 during treatment with the vitamins D*

Patient No./sex/age	Study period	Dose of vitamin D metabolite (ng/kg/day)			Calcium % retention		Intake (mmol/d)
		$1,25(OH)_2D_3$	$24,25(OH)_2D_3$	$25(OH)D_3$	Control period	Treatment period	
1.H.J.,F,26	(a)	—	—	—	21		29.6
	(b)	8	—	—	—	36	,,
	(c)	8	80	—	—	41	27.7
2.P.M.,F,12	(a)	—	—	—	18	—	27.7
	(b)	9	—	—	—	22	,,
	(c)	9	370	—	—	25	,,
3.S.S.,F,19	(a)	—	—	—	2	—	59.0
	(b)	11	—	—	—	27	,,
	(c)	11	210	—	—	29	,,
	(d)	—	—	842	—	45	,,
4.C.V.,F,28	(a)	41	202	—	—	45	57.9
	(b)	—	—	810	—	34	,,
5.M.D.,F,35	(a)	42	—	—	—	36	59.6
	(b)	—	—	837	—	53	59.2
6.P.V.,F,21	(a)	58	—	—	—	60	58.7

25OHD (Haussler and McCain, 1977). It suggests that the relative ineffectiveness of $1,25(OH)_2D_3$ in healing rickets (though not in producing vitamin D-intoxication) may be due to the unphysiological effect of bolus administration or that $25OHD_3$ itself (or a derivative other than $24,25(OH)_2D_3$) may exert a separate effect, for example, to stimulate renal tubular phosphate handling. This view is so far consistent with the data of Bordier et al (1978) and a careful study of phosphorus metabolism is therefore warranted.

Figure 5 (patient 5) shows the marked superiority of $25OHD_3$ over $1,25(OH)_2D_3$ in raising plasma phosphorus levels (by increasing renal tubular phosphate reabsorption), a separate event from (prolonged) mineral retention in the skeleton. Moreover this rise was produced despite a steep simultaneous reduction in her phosphorus intake achieved by substituting an equivalent soluble calcium supplement for microcrystalline hydroxyapatite. Patient 2 developed frank hyperphosphataemia (serum phosphorus 1.68 mmol/l) 11 days after substituting $25OHD_3$. If these changes were consistent they would plainly support an effect of $25OHD_3$ that was not shared by $1,25(OH)_2D_3$; but unfortunately they are not. Not only may they be absent

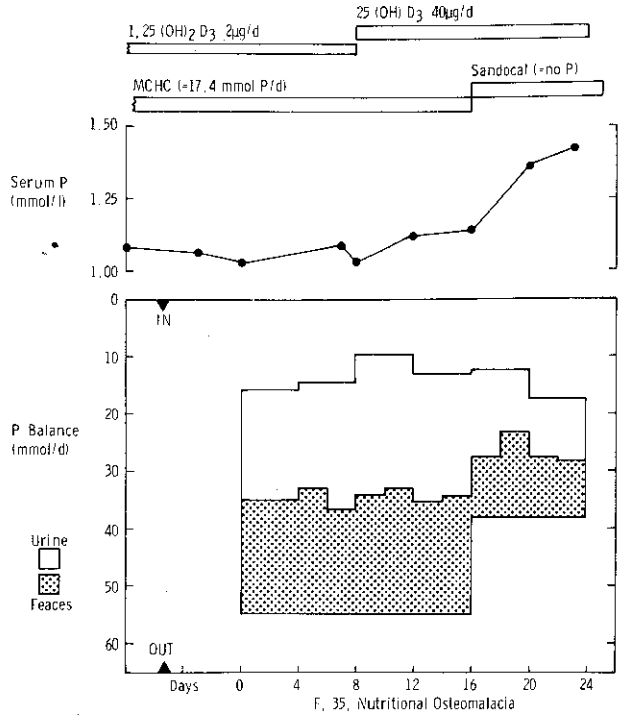

Figure 5. Phosphorus balance in patient 5 (Table 1). Note the marked superiority of $25(OH)D_3$ over $1,25(OH)_2D_3$ in raising serum phosphorus despite simultaneous reduction in phosphorus intake (see text). The serum phosphorus rise was associated with reduction in urine phosphorus excretion.

(patient 4 showed diminishing positive phosphorus balance and unchanged serum phosphorus on $25OHD_3$) but $1,25(OH)_2D_3$ alone may fully enhance renal tubular phosphate reabsorption. One patient (Stamp et al, 1979: K.G., Table 2) showed a rise in serum phosphorus from 0.78 mmol/l (mean pre-treatment level) to 1.44 mmol/l with a fall of 8.6 mmol/day in urine phosphorus after beginning $1,25(OH)_2D_3$ 2 μg/day. In order to exclude the possibility that apparent effects of $1,25(OH)_2D_3$ (with or without $24,25(OH)_2D_3$) were influenced by accidental improvement in vitamin D supply we repeated 25OHD determinations at the end of all these studies. There was a mean rise in five patients of 0.8 ng/ml after treatment averaging 10 weeks' duration; in a sixth patient the 'change' was from 3.2 to '<4.1' ng/ml.

While strict analysis of the correlation between changes in renal tubular phosphate reabsorption and in calcium retention may be called for, in general terms it appears that the higher the early rise in plasma phosphorus the stronger the external calcium balance *however this is achieved.* The therapeutic superiority of $25OHD_3$ over $1,25(OH)_2D_3$ *in osteomalacia*, relative to their respective normal plasma levels and, more importantly, to their powers of vitamin D intoxication in other clinical situations, still requires explanation.

LONG-TERM CHANGES IN PHOSPHORUS AND ALKALINE PHOSPHATASE

Serum phosphorus is primarily governed by renal tubular reabsorption for which a T_{max} exists (Pitts and Alexander, 1944; Hiatt and Thompson, 1957a,b; Thompson and Hiatt, 1957a, b; Bijvoet, 1969; Stamp and Stacey, 1970). The rise in serum phosphorus during treatment of rickets or osteomalacia is associated with a rise in TmP when this has been measured directly by phosphorus infusions (Dent and Stamp, 1970). It may rarely be profound (Stamp and Stacey, 1970). Short-term studies of serum phosphorus changes too numerous to detail from the literature have long suggested that phosphorus may rise *above normal* at the early healing stages. However, few studies have confirmed frank hyperphosphataemia and the extent or significance of this possible change is still uncertain. We therefore followed serum biochemical changes during treatment in 41 patients of whom 21 had measurements charted for at least seven to 12 months. Observation suggested that at least six months' continuous treatment was required for complete healing and we assumed, for the purposes of this study, that measurements after seven to 12 months' treatment represented normal values for each individual. In order to give equal weight to rapid short-term changes and to slow long-term changes we arbitrarily selected the following time intervals after beginning effective treatment: 4-8 days, 9-16 days, 17-32 days, 5-9 weeks, 10-15 weeks, 4-6 months, 7-12 months and 13 months or over. Values for serum calcium, phosphorus and phosphatase in each patient were recorded, when available, within appropriate periods for purposes of comparison. If more than one value from any individual had been obtained in a given period a mean was taken.

Results and Discussion

Phosphorus

Plasma phosphorus changes in 21 adult patients are presented in Figure 6 as the mean differences from each patient's value after seven to 12 months' continuous treatment. Each point, with bars indicating the limits of 2 × s.e.m., therefore shows the calculations which provided separate paired t-tests against the 'baseline' (seven to 12 months). The *P* values shown were obtained from these separate paired t-tests. Certain random bias was introduced by the fact that not every patient was sampled during every time-interval (numbers are shown by N in Figure 6). Although this statistical treatment might be expected to tend to smooth out the observed changes,

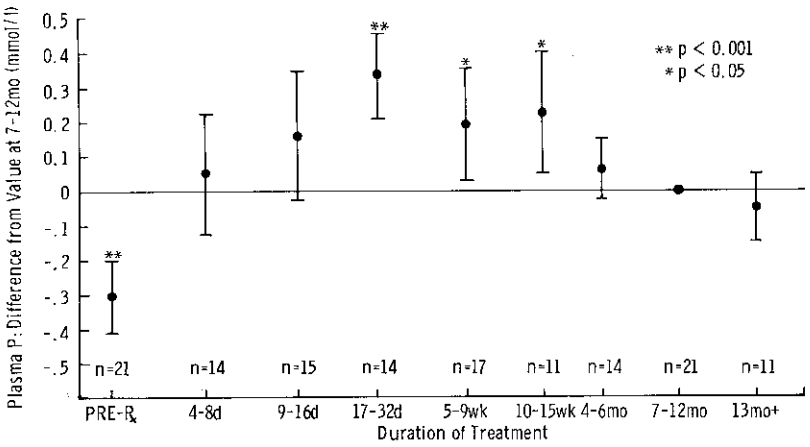

Figure 6. Mean differences in serum phosphorus, before and at various periods after starting treatment, from individual levels recorded after 7 to 12 months' continuous treatment in 21 adults with nutritional osteomalacia. Bars represent limits of 2 × s.e.m. and were used to calculate separate paired t-tests against the 7 to 12 month baseline (*P* values derived from these t-tests). Note that patients showed a highly significant biphasic response illustrated in this plot of duration of treatment on a log time-scale.

data show conclusively for the first time that plasma phosphorus responds biphasically, the first phase being an *abnormal* rise in the population as a whole: this is sustained for four to six months before settling to the normal physiological level. Since the normal fall in serum phosphorus at the end of adolescence might artefactually influence these data, results in adolescents were omitted; changes in three adolescent males are shown separately in Figure 7 on a log-time scale and illustrate also how the data from Figure 6 and 8 were obtained. Long-term follow-up was plainly required to confirm this pattern. Table 2 shows results in the other 21 patients whose follow-up was not complete beyond four to six months, with phosphorus levels necessarily represented as change from pre-treatment values; the significant

secondary fall was not clear. All patients were on vitamin D supplements during their follow-up usually, but not always, consisting of capsules of vitamins A and D, B.N.F., two twice daily providing 1800 iu vitamin D daily. This treatment produces a mean 25OHD level of 22 ng/ml (after log transformation and reconversion) with a range 11-46 ng/ml (± 2 × s.d. of log data) as has previously been shown (Stamp, Haddad and Twigg, 1977).

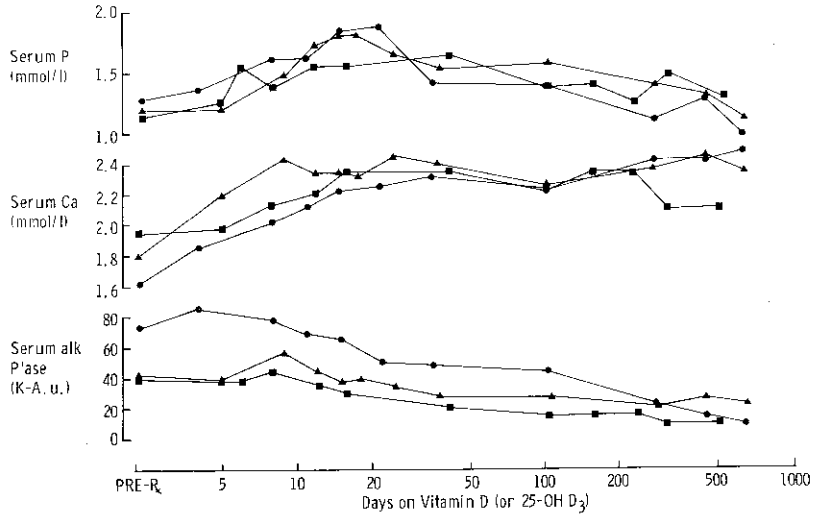

Figure 7. Serum calcium, phosphorus and alkaline phosphatase in three adolescent males during long-term treatment of nutritional osteomalacia. Data were plotted on a log time-scale in order to give equal emphasis to rapid short-term changes and slower long-term changes (the same axis is used in Figure 6).

Total serum calcium rose quickly to normal, stable levels (Table 3) suggesting that the prolonged phosphate effect was not mediated by calcium ions alone. Since the mean rise in calcium from pre-treatment values at 10 to 15 weeks in 10 further patients (again excluding hypocalcaemic adolescents) was + 0.23 mmol/l, the apparently significant fall in calcium at this time in Table 3 is likely to be accidental.

The increase in Ca × P solubility product produced by a separate effect of vitamin D enhancing renal tubular phosphate reabsorption above normal physiological levels is likely to be advantageous to re-mineralization of osteomalacic bone. It is clearly prolonged throughout most, if not all, of the latter process and raises the question, which seems an important one, of possible mechanism. The source of a possible 'message' to the renal tubule is not apparent. Further work is required on its possible relationship to the time-scale of involution of secondary hyperparathyroidism, for example, and of the tropic effect of parathyroid hormone on the utilization of 25(OH)D_3 (Stanbury and Mawer, 1978a,b).

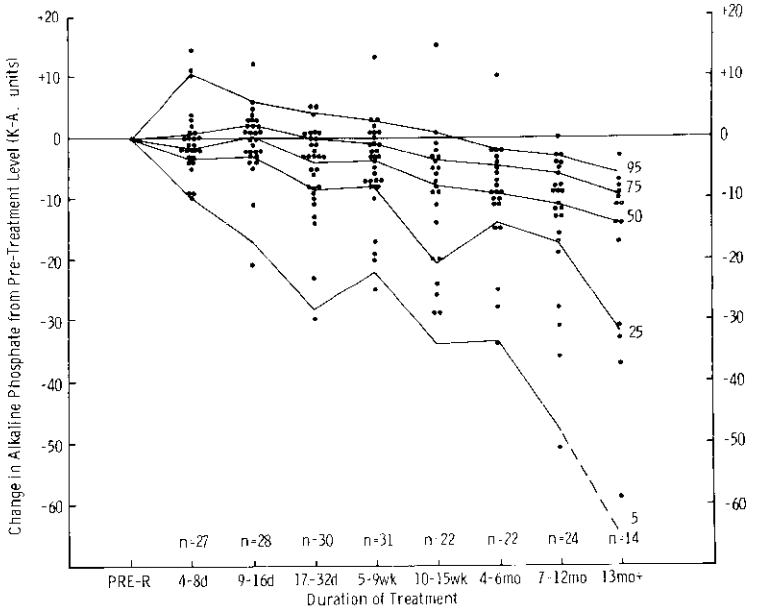

Figure 8. Change in serum alkaline phosphatase from pre-treatment levels among 41 patients with nutritional osteomalacia and late rickets. Changes during treatment were grouped within one of the eight time-periods shown. Percentiles were calculated from the cumulative frequency distribution of the sample data for each period.

Alkaline phosphatase

Raised alkaline phosphatase shows a skewed distribution which can be corrected by logarithmic representation (Morgan, 1973). Many workers have noted an occasional further rise in early healing (Morgan et al, 1965) but attempts to quantitate the scale of these changes, both in relation to size and to frequency, have not previously been made. We followed the change in serum alkaline phosphatase levels in all 41 of our patients whose treatment had been monitored from the beginning. Individual changes from pre-treatment levels were again represented within the time periods described above (see Figure 8). Percentiles were calculated from the cumulative

Table 2. *Mean differences in serum phosphorus from individual pre-treatment levels in 17 adults with osteomalacia (n = number of patients with values recorded in each time-period)*

Duration of treatment	4-8 days	9-16 days	17-32 days	5-9 weeks	10-15 weeks	4-6 months
Mean difference in serum phosphorus from pre-treatment level (mmol/l)	+0.16	+0.26	+0.32	+0.27	+0.26	+0.20
2 × s.e.m.	0.11	0.11	0.11	0.16	0.15	0.24
n	10	10	13	12	10	6
t value	3.06	4.56	5.73	3.53	3.57	1.65

Table 3. *Mean differences in serum calcium, before and at various periods after starting treatment, from levels recorded after 7-12 months' continuous treatment in 21 adults with nutritional osteomalacia (n = number of patients with values recorded in each time-period)*

Duration of treatment	Pre-	4-8 days	9-16 days	17-32 days	5-9 weeks	10-15 weeks	4-6 months	7-12 months	13 months +
Mean difference in serum calcium from value at 7-12 months (mmol/l)	−0.18	−0.03	−0.03	+0.02	+0.03	−0.07	−0.03	0	+0.05
2 × s.e.m.	0.07	0.08	0.07	0.06	0.06	0.06	0.03	—	0.05
n	21	13	13	14	17	12	14	21	11

frequency distribution of the sample data for each time period. Although, again, changes may be smoothed out by this form of representation and random bias is introduced by the different frequency when different patients were sampled, data indicate that over 25 per cent of patients may show an alkaline phosphatase 'flare', which in a smaller minority reaches above 10 K-A units and which may be sustained for at least 17 to 32 days. The variable biphasic change in alkaline phosphatase contrasts with the constant change in phosphorus.

The physiological role of alkaline phosphatase is still uncertain, as is its relationship to osteoblastic activity (Felix and Fleisch, 1977; Aaron, 1978; Posen, 1979). Further work is therefore required to establish, for example, whether the alkaline phosphatase 'flare' represents a temporary positive resultant between renewed activity of previously-inactive osteoblasts (which come to cover the widened osteoid seams) and the healing process.

SUMMARY

Despite a century of scientific study and of socioeconomic advance classical rickets and osteomalacia are as apparent in England today as in the developing world.

Forty-five patients with overt classical late rickets and osteomalacia presenting over a five-year period provided the basis for a study of age, sex and race distribution, symptoms and their onset, clinical signs, radiology and response to treatment. Results, together with measurements of plasma 25OHD among newly arrived emigrants from Uganda, suggest that residence in England is a significant factor in the production of disease among Asian patients originating from East Africa.

Biochemical abnormalities were quantitated. Fifty per cent of patients with overt disease were normocalcaemic. Serum alkaline phosphatase may be unequivocally normal in severe disease. Human requirement for oral $1,25(OH)_2D_3$ when replacing outside sources of vitamin D was close to 7 ng/kg/day. $24,25(OH)_2D_3$ was neither effective nor necessary for the apparent cure of osteomalacia. $1,25(OH)_2D_3$ was no more than 10 to 40 times more effective than $25(OH)D_3$ on a weight basis. Both $1,25(OH)_2D_3$ and $25(OH)D_3$ effectively increased renal tubular phosphate reabsorption.

Serum phosphorus showed a highly significant biphasic response during long-term treatment, elevated levels being recorded during most of the period of bone remineralization (up to four months). The extent and frequency of a rise in serum alkaline phosphatase during treatment was quantitated.

ADDENDUM

Plasma bicarbonate levels were measured in 13 consecutive patients after at least 6 months' effective treatment and showed a highly significant mean rise of 3.0 ± 1.7 mmol/l ($P<0.001$ or paired values) from pre-treatment levels. These findings, in patients whose vitamin D-deficiency was not complicated by malabsorption syndrome or renal disease, confirm that classical osteomalacia is associated with mild metabolic acidosis.

Liu, S. H., Hannon, R. R., Chu, H. I., Chen, K. C., Chou, S. K. & Wang, S. H. (1935) Calcium and phosphorus metabolism in osteomalacia. II Further studies on the response to vitamin D of patients with osteomalacia. *Chinese Medical Journal*, **49**, 1-21.

Loomis, W. F. (1970) Rickets. *Scientific American*. **223**, 77-91.

Looser, E. (1920a) Über Spätrachitis und Osteomalacie. Klinische, rontgenologische und pathologischanatomische Untersuchungen. *Deutsche Zeitschrift für Chirurgie*, **152**, 210-357.

Looser, E. (1920b) Über Pathologische Formen von Infractionen und Callusbildungen bei Rachitis und Osteomalakie und anderen Knochenerkrankungen. *Zentrablatt fur Chirurgie*, **47**, 1470-1474.

McLaughlin, M., Fairney, A., Lester, E., Raggatt, P. R., Brown, D. J. & Wills, M. R. (1974) Seasonal variations in serum 25 hydroxycholecalciferol in healthy people. *Lancet*, **i**, 536-538.

Maxwell, J. P., Pi, H. T., Liu, H. A. C. & Kuo, C. C. (1939) Further studies in adult rickets (osteomalacia) and foetal rickets. *Proceedings of the Royal Society of Medicine*, **32**, 287-297.

Morgan, D. B. (1973) The osteomalacia syndrome due to vitamin D deficiency. In *Osteomalacia, Renal Osteodystrophy and Osteoporosis* (Ed.) Kugelmass, I. N. pp. 73-144. Springfield, Illinois: C. C. Thomas.

Morgan, D. B., Hunt, G. & Paterson, C. R. (1970) The osteomalacia syndrome after stomach operations. *Quarterly Journal of Medicine*, **39**, 395-410.

Morgan, D. B., Paterson, C. R., Woods, C. G., Pulvertaft, C. N. & Fourman, P. (1965) Osteomalacia after gastrectomy; responses to very small doses of vitamin D. *Lancet*, **ii**, 1089-1091.

Muldowney, F. P., Freaney, R. & McGeeney, D. (1968) Renal tubular acidosis and amino-aciduria in osteomalacia of dietary or intestinal origin. *Quarterly Journal of Medicine*, **37**, 515-528.

Norman, A. W. & Henry, H. L. (1979) Both 24R, 25-dihydroxyvitamin D_3 and 1α 25-dihydroxy-vitamin D_3 are indispensable for normal calcium and phosphorus homeostasis. In *Vitamin D Basic Research and its Clinical Application* (Ed.) Norman, A. W., Schaefer, K., Herrath, D., Grigoleit, H-G., Coburn, J. W., DeLuca, H. F., Mawer, E. B. & Suda, T. pp. 571-578. Berlin: de Gruyter.

Ornoy, A., Goodwin, D., Noff, D. & Edelstein, S. (1978) 24,25-Dihydroxyvitamin D is a metabolite of vitamin D essential for bone formation. *Nature* (London), **276**, 517-519.

Palm, T. A. (1980) The geographical distribution and aetiology of rickets. London. *Practitioner*, **xiv**, 270-279, 321-342.

Paterson, C. R. (1973) Osteomalacia and rickets. In *Metabolic Disorders of Bone*. pp. 165-191. Oxford: Blackwell.

Pommer, G. (1885) *Untersuchungen über Osteomalacie und Rachitis*. Leipzig: Vogel.

Pitts, R. F. & Alexander, R. S. (1944) The renal reabsorptive mechanism for inorganic phosphate in normal and acidotic dogs. *American Journal of Physiology*, **142**, 648-662.

Posen, S. (1979) Do alkaline phosphatases have a physiological function? In *Vitamin D. Basic Research and Clinical Application* (Ed.) Norman, A. W., Schaefer, K., v. Herrath, D., Grigoleit, H-G., Coburn, J. W., DeLuca, H. F., Mawer, E. B. & Suda, T. pp. 983-990. Berlin: de Gruyter.

Preece, M. A., McIntosh, W. B., Tomlinson, S., Ford, J. A., Dunnigan, M. G. & O'Riordan, J. L. H. (1973) Vitamin D deficiency among Asian immigrants in Britain. *Lancet*, **i**, 907-910.

Rosin, A. J. (1970) Clinical features and detection of osteomalacia in the elderly. *Postgraduate Medical Journal*, **46**, 131-136.

Smith, I., Seakins, J. W. T. & Dayman, J. (1969) *Chromatographic and Electrophoretic Techniques*, Volume 1, 3rd edition (Ed.) Smith, I. p. 364. London: Heinemann.

Smith, R. (1979) *Biochemical Disorders of the Skeleton*. p. 48. London: Butterworths.

Stamp, T. C. B. (1975) Factors in human vitamin D nutrition and in the production and cure of classical rickets. *Proceedings of the Nutrition Society*, **34**, 119-130.

Stamp, T. C. B. (1980) Sources of Vitamin D nutrition. *Lancet*, **i**, 316.

Stamp, T. C. B. & Stacey, T. E. (1970) Evaluation of theoretical renal phosphorus threshold as an index of renal phosphorus handling. *Clinical Science*, **39**, 505-516.

Stamp, T. C. B. & Round, J. M. (1974) Seasonal changes in human plasma levels of 25-hydroxy-cholecalciferol. *Nature* (London), **247**, 563-565.

Stamp, T. C. B., Haddad, J. G. & Twigg, C. A. (1977) Comparison of oral 25-hydroxycholecalciferol, vitamin D and ultraviolet light as determinants of circulating 25-hydroxyvitamin D. *Lancet*, **i**, 1341-1343.

Stamp, T. C. B., Perry, W., MacArthur, S. & Jenkins, M. V. (1979) Treatment of privational late rickets and osteomalacia with the vitamins D. *Vitamin D Basic Research and Clinical Application* (Ed.) Norman, A. W., Schaefer, K., v. Herrath, D., Grigoleit, H-G., Coburn, J. W., DeLuca, H. F., Mawer, E. B. & Suda, T. pp. 1153-1162. Berlin: de Gruyter.

Stanbury, S. W. & Mawer, E. B. (1978a) Physiological aspects of vitamin D metabolism in man. In *Vitamin D* (Ed.) Lawson, D. E. M. pp. 303-341. London: Academic Press.

Stanbury, S. W. & Mawer, E. B. (1978b) Clinical aspects of vitamin D metabolism in man. In *Vitamin D* (Ed.) Lawson, D. E. M. pp. 343-386. London: Academic Press.

Thompson, D. D. & Hiatt, H. H. (1957a) Renal reabsorption of phosphate in normal human subjects and in patients with parathyroid disease. *Journal of Clinical Investigation*, **36**, 550-556.

Thompson, D. D. & Hiatt, H. H. (1957b) Effects of phosphate loading and depletion on renal excretion and reabsorption of inorganic phosphate. *Journal of Clinical Investigation*, **36**, 566-572.

Vainsel, M., Manderlier, T. & Vis, H. L. (1974) Proximal renal tubular acidosis in vitamin D deficiency rickets. *Biomedicine*, **22**, 35-40.

Vainsel, M., Manderlier, T., Corvilain, J. & Vis, H. L. (1974) Study of hyperparathyroidism in vitamin D deficiency rickets. II Aspects of aminoacid metabolism. *Biomedicine*, **20**, 404-409.

Vaishnava, H. P. (1975) Vitamin D deficiency osteomalacia in Northern India. *Journal of the Association of Physicians of India*. **23**, 477-484.

Vaishnava, H. & Rizvi, S. N. A. (1971) Nutritional osteomalacia in immigrants in an urban community. *Lancet*, **ii**, 1147-1148.