Ann Clin Biochem 1987; **24**: 22–28

Calcium, vitamin D and parathyroid hormone relationships in pregnant Caucasian and Asian women and their neonates

F OKONOFUA, R K MENON, S HOULDER, M THOMAS, D ROBINSON, S O'BRIEN and P DANDONA

From the Departments of Chemical Pathology and Human Metabolism, and Obstetrics and Gynaecology, Royal Free Hospital and School of Medicine, London NW3 2QG, UK

SUMMARY. Plasma calcium, serum 25 hydroxyvitamin D [25(OH)D], 1,25 dihydroxyvitamin D[1,25$(OH)_2$D] and parathyroid hormone (PTH) have been measured in pregnant and newborn Caucasians and Asians. Calcium and 25(OH)D concentrations were lower in Caucasian than in Asian women at all four stages (three trimesters and during labour) of pregnancy. PTH concentrations were greater in Asian than in Caucasian women during the three trimesters, but not at labour, and increased in both groups through pregnancy, without a concomitant change in plasma calcium concentrations. There was a significant inverse correlation between calcium and PTH, as well as 25(OH)D and PTH, concentrations. These data demonstrate the presence of progressive 'hyperparathyroidism' during pregnancy in Caucasian and Asian women. The higher PTH concentrations in Asian women may reflect the necessity of maintaining adequate plasma calcium concentrations through PTH-induced osteolysis in the face of vitamin D deficiency. Relative hyperparathyroidism in Asians may contribute to net loss of calcium from the skeleton and osteopenia in Asian women. Calcium, 25(OH)D and 1,25$(OH)_2$D concentrations were lower, and those of PTH higher, in Asian newborns compared with Caucasian newborns. Serum 1,25$(OH)_2$D concentrations in the Asian newborn, though lower than respective maternal levels, were comparable with normal adult levels, indicating that 1,25$(OH)_2$D biosynthesis is stimulated in the Asian newborn to compensate for the low serum 25(OH)D concentrations.

It has been demonstrated that asymptomatic Asian immigrants in the United Kingdom may have subclinical hypovitaminosis D and secondary hyperparathyroidism.[1–3] It has also been shown that the concentrations of 25 hydroxyvitamin D [25(OH)D] in cord blood are significantly lower than those in maternal serum.[4–6] These facts raise the possibility that secondary hyperparathyroidism may be frequent in Asian women during pregnancy, and that these women's infants may also have secondary hyperparathyroidism. It has previously been suggested that serum concentrations of PTH increase during pregnancy;[7,8] this observation has, however, been challenged by other workers.[9,10] To our knowledge, there is only one previous study which has compared 25(OH)D and PTH concentrations in the serum of pregnant Caucasian and Asian women.[11] In this study, radioimmunoassays of PTH were carried out by the method of Rosselin *et al.*,[12] and failed to detect any differences in serum PTH between Asian and Caucasian women. Since we and others, using more recent radioimmunoassay methodology,[1–3] have reported frequent secondary hyperparathyroidism in non-pregnant Asians, it is clear that 25(OH)D-calcium-PTH relationships during pregnancy need to be re-examined. Finally, no data are available about 1,25 dihydroxyvitamin D [1,25$(OH)_2$D] concentrations in Asian and Caucasian women at term and their newborns.

Correspondence: Dr P Dandona, Metabolic Unit, Department of Chemical Pathology and Human Metabolism, Royal Free Hospital, Pond Street, London NW3 2QG, UK.

This study was instituted to determine whether a progressive increase in PTH concentrations occurs during pregnancy, whether such an increase is more marked in Asian women with hypovitaminosis D, whether a progressive fall in 25(OH)D concentrations occurs in Asian women with vitamin D deficiency due to increased requirements associated with the pregnant state, and whether the lower 25(OH)D concentrations in the Asian pregnant woman result in lower 1,25 $(OH)_2D$ concentrations in the woman herself and in her newborn.

Patients and methods

Forty-three Asian and 55 Caucasian pregnant women attending the Antenatal Clinic at The Royal Free Hospital, London, during the months of September to November were investigated. For the purposes of this study, 'Asians' are people from the Indian subcontinent, and 'Caucasians' are white-skinned people from the British Isles. Approval for the study was obtained from the hospital's Ethical Committee. Written, informed consent was solicited individually from the patients before they were included in the study. There was no selection of cases and all the patients who agreed to participate in the fully explained project were included. They were told that they would be treated with vitamin D if any deficiency of the vitamin was detected.

No change was made in the diet of any of the patients during pregnancy and no supplemental vitamins had been prescribed prior to collection of blood samples for this study. Thirty-five Asians were strict vegetarians, whilst all the Caucasians were non-vegetarians.

Blood samples were collected at various stages of pregnancy, as shown in Table 1.

On each occasion, blood was collected into two tubes, one heparinised for plasma separation and the other for serum. Serum and plasma samples were separated within 30 min of collection. The serum samples were separated using a refrigerated centrifuge and immediately deep-frozen, so that all the 25(OH)D, 1,25$(OH)_2D$ and PTH assays could be performed in batches. To minimise interassay variation, we carred out all estimations of 25(OH)D and PTH in three batches, making sure that all Asian and Caucasian samples from a particular period during pregnancy were assayed together.

TABLE 1. Collection of blood samples

	Asian (n=43)	Caucasian (n=55)
Period I: 10–20 weeks	11	18
Period II: 21–30 weeks	11	10
Period III: 31–40 weeks	10	15
Period IV: Paired maternal (during labour) and newborn (umbilical vein) samples	11	12

The serum concentration of 25(OH)D was measured by a modification of Preece *et al.*,[13] which involves preparatory chromatography followed by a protein binding step. The minimal consistently detectable concentration of 25(OH)D in this assay is 5 nmol/L.

Serum PTH concentrations were determined by a radioimmunoassay procedure using an antibody directed against the mid-molecular peptide of PTH.[14, 15] As previously published, the interassay variation for this estimation is 12%, whilst the intra-assay variation is less than 8%.[16] Serum 1,25 $(OH)_2D$ was assayed by a method utilising an initial chromatographic separation step followed by binding to a specific receptor prepared from bovine thymic extracts.[17] The interassay variation for this method is 12%, whilst intra-assay variation is less than 8%. The reagents used in these assays were obtained from Immunonuclear Corporation, Stillwater, Minnesota, USA.

Aliquots of heparinised plasma were used for the measurements of calcium, phosphate, alkaline phosphatase, magnesium and albumin on a SMAC Technicon Auto analyser.

Statistical analysis was carried out by the Mann-Whitney U test.

Results

Plasma calcium concentrations did not alter significantly during pregnancy in either Asian or Caucasian women. However, they were considerably lower in Asian than in Caucasian women at each of the four stages of pregnancy (Table 2). Calcium concentrations in all women were within the normal reference range (2·10–2·60 mmol/L) for our laboratory.

Plasma phosphate concentrations were also significantly lower in Asian women during the first and third trimester and during labour (Table 2). Plasma alkaline phosphatase, albumin and magnesium concentrations were almost indentical in Asian and Caucasian

women at all four stages of pregnancy (Table 2).

Compared with Caucasian women, serum 25(OH)D concentrations were markedly lower in Asian women at all four stages of pregnancy. There was no evidence of a significant change in 25(OH)D concentrations in either Asian or Caucasian women during pregnancy (Table 2).

PTH concentrations increased steadily throughout pregnancy in Caucasian women, the median PTH concentrations during the second and third trimesters and during labour being significantly greater than those during the first trimester (Fig. 1). Asian women also had a significant increase in PTH between the first and third trimesters, but there was no further increase during labour. PTH concentrations were significantly greater in Asian women than those in Caucasians during the first, second and third trimesters. PTH concentrations during labour were also higher in Asians than those in Caucasians, but the difference was not statistically significant (Fig. 1). There was a highly significant inverse correlation between plasma calcium and PTH concentrations in pregnant women (Fig. 2); there was also a significant inverse correlation between 25(OH)D and PTH in these women (Fig. 3). When Asian and Caucasian women were considered separately, the inverse correlation between calcium and PTH persisted (r=0·41 for both Asians and Caucasians; P<0·002), whilst that between 25(OH)D and PTH disappeared.

1,25$(OH)_2$D concentration during labour

TABLE 2. Plasma calcium, inorganic phosphorus, alkaline phosphatase, albumin, magnesium and 25 OH vitamin D [25(OH)D] in Asian and Caucasian women in the four sampling periods. Results are shown as median and (range)

	Period I	Period II	Period III	Period IV
Calcium (mmol/L)				
Caucasian	2.36 (2·28–2·53)	2·39 (2·31–2·51)	2·36 (2·10–2·51)	2·32 (2·27–2·72)
Asian	2·29 (2·13–2·45)	2·30 (2·20–2·35)	2·30 (2·10–2·41)	2·27 (1·91–2·33)
P value	<0·002	<0·002	<0·02	<0·002
Inorganic Phosphorus (mmol/L)				
Caucasian	1·21 (0·91–1·4)	1·13 (0·88–1·3)	1·12 (0·79–1·28)	1·16 (0·82–1·60)
Asian	1·16 (0·78–1·22)	1·09 (0·97–1·19)	1·07 (0·87–1·21)	1·07 (0·89–1·25)
P value	<0·002	NS	<0·02	<0·02
Alkaline phosphatase (i.u./L)				
Caucasian	50 (33–137)	75 (44–135)	141 (88–216)	228 (180–190)
Asian	47·5 (40–93)	78 (39–88)	146 (61–200)	231 (165–297)
P value	NS	NS	NS	NS
Albumin (g/L)				
Caucasian	36 (30–42)	35 (32–38)	32 (30–35)	33 (30–42)
Asian	34 (31–38)	34 (31–39)	32 (30–34)	32·5 (30–35)
P value	NS	NS	NS	NS
Magnesium (mmo/L)				
Caucasian	0·75 (0·60–0·88)	0·75 (0·67–0·82)	0·74 (0·62–0·85)	0·71 (0·56–0·82)
Asian	0·76 (0·62–0·82)	0·71 (0·65–0·78)	0·73 (0·63–0·87)	0·71 (0·54–0·85)
P value	NS	NS	NS	NS
25(OH)D (nmol/L)				
Caucasian	21·7 (11·0–57·2)	42·5 (14·7–64·5)	26·5 (5·0–39·5)	23·5 (14·5–46·2)
Asian	6·2 (<5·0–8·2)	12·5 (<5·0–18·7)	7·8 (<5·0–26·5)	13·1 (<5·0–28·5)
P value	<0·002	<0·002	<0·002	<0·002

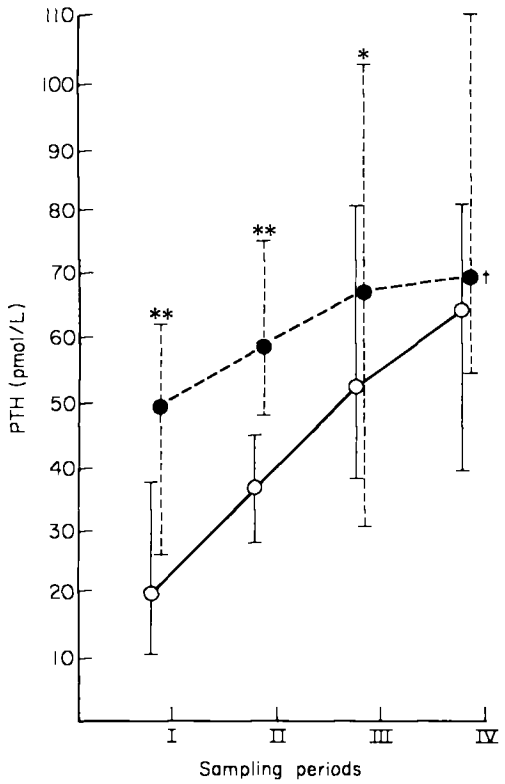

FIG. 1. Progressive increase in PTH concentrations during pregnancy in Caucasian (○——○) and Asian women (●— — —●). Data are medians and ranges; *$P<0.05$; **$P<0.002$; †NS.

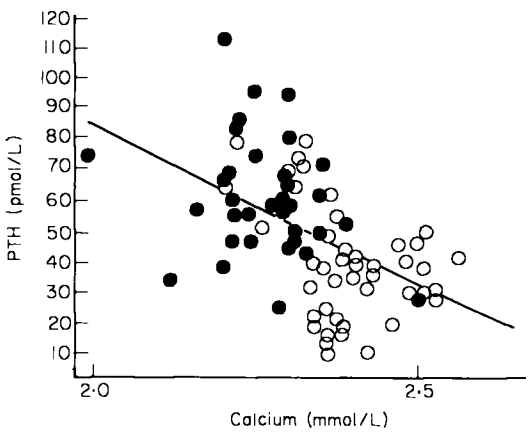

FIG. 2. Inverse correlation between maternal calcium and PTH concentrations ($r=0.51$; $P<0.0001$). Asians: ●; Caucasians: ○.

was significantly lower in Asian women than in their Caucasian counterparts (Table 3). The concentration of this metabolite was also significantly lower in Asian newborns than in Caucasian newborns. PTH concentrations in Asian newborns were higher and 25(OH)D concentrations significantly lower than those in

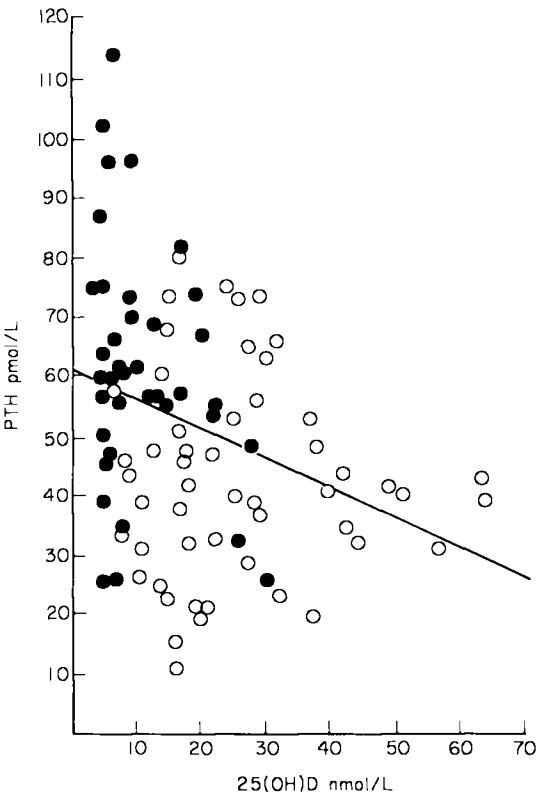

FIG. 3. Inverse correlation between serum 25(OH)D and PTH concentrations in maternal serum ($r=0.32$; $P<0.002$). Asians: ●; Caucasians: ○.

TABLE 3. Serum 1,25 dihydroxyvitamin D concentration in Asian and Caucasian women at term and in their newborns. Results are expressed as median and (range) (pmol/L). The normal range for non-pregnant Caucasian women is 40–120 pmol/L

	Maternal	Cord
Caucasian	161 (79–335)	104 (48–214)
Asian	107 (41–243)	83 (51–108)

Caucasian *vs* Asian mothers: $P<0.05$; Caucasian *vs* Asian cords: $P<0.01$; Caucasian maternal *vs* cords: NS; Asian maternal *vs* cords: $P<0.05$.

TABLE 4. Plasma calcium, inorganic phosphorus, magnesium, alkaline phosphatase, 25 hydroxyvitamin D [25(OH)D] and parathyroid hormone (PTH) in cord blood (Asian and Caucasian)

	Calcium (mmol/L)	Inorganic phosphorus (mmol/L)	Alkaline phosphatase (i.u./L)	Magnesium (mmol/L)	PTH (pmol/L)	25(OH)D (nmol/L)
Asian cord blood	2·65 (2·43–2·98)	1·50 (1·43–1·84)	126 (116–282)	0·79 (0·66–0·97)	55 (34–95)	6·2 (<5·0–25·0)
Caucasian cord blood	2·76 (2·56–2·89)	1·48 (1·21–1·79)	168 (120–274)	0·78 (0·62–0·93)	44 (22–58)	15·3 (6·4–39·0)
P value	0·02	NS	NS	NS	<0·05	<0·05

Caucasian newborns (Table 4). $1,25(OH)_2D$ concentrations in all newborns were lower than those observed in their respective mothers at term.

Discussion

Our data have answered clearly all the questions which were set out earlier. In neither Caucasian nor Asian pregnant women was there a significant decrease in 25(OH)D concentrations, despite the increased requirements of vitamin D during pregnancy. The concentration of $1,25(OH)_2D$ at term was significantly greater than that seen in non-pregnant women. However, the $1,25(OH)_2D$ concentrations in Asian women were significantly lower than those observed in Caucasians. It is noteworthy that Asian women were able to increase their $1,25(OH)_2D$ concentrations during pregnancy in spite of extremely low 25(OH)D concentrations, and that Asian newborns also generated significant $1,25(OH)_2D$ concentrations, although some had undetectable 25(OH)D. The lower 25(OH)D and $1,25(OH)_2D$ concentrations in Asian women were reflected in the significantly elevated concentrations of PTH in serum of Asian women and significantly lower plasma calcium and phosphate concentrations in this group when compared with those of Caucasians. There was a progressive increase in PTH concentrations during pregnancy in both Asians and Caucasians. This increase was, however, more marked in Caucasians (Fig. 1) These data are consistent with a state of progressive hyperparathyroidism in pregnancy which is independent of any significant changes in plasma calcium concentrations. Whether the increase in PTH concentrations is aimed at stimulating the production of $1,25(OH)_2D$ or at greater mobilisation of calcium from the skeleton or at conserving renal losses of calcium to make more calcium available for foetal growth is not clear. It is also possible that PTH increases to compensate for a possible decrease in sensitivity to PTH during pregnancy. Oestrogens may have a modulating effect on the end organ responses to PTH. Furthermore, calcitonin concentrations are known to increase during pregnancy[18] and the persistent calcium lowering effect of calcitonin may induce relative resistance to parathyroid hormone. Finally, severe vitamin D deficiency may induce relative resistance to PTH[19] to an extent that hyperphosphataemia and hypophosphaturia may occur;[20,21] this would be relevant to Asian women and infants.

One important implication of progressive secondary hyperparathyroidism in Asian women during pregnancy is the possible effect of hyperparathyroidism on bones. We have recently reported the occurrence of pathological fractures in two Asian women during the third trimester of pregnancy.[22] This may have been contributed to by the concomitant secondary hyperparathyroidism.

The demonstration of a significant inverse relationship between calcium and PTH concentrations in a group of women whose calcium concentrations are within the normal range is of interest and has, to our knowledge, not been shown before. Clearly, therefore, PTH secretion responds to alterations in calcium concentrations during pregnancy, irrespective of the underlying trend towards an increase in PTH concentrations during pregnancy.

The finding that the markedly lower concentrations of 25(OH)D in Asian women are associated with only a minimal increase in PTH concentrations when compared with Caucasian women probably reflects the fact that Asian women are able to 'compensate' to some extent by increasing the production of $1,25(OH)_2D$. The data on women at term (stage IV) indicates this, but since the measurements of $1,25(OH)_2D$

during the earlier part of pregnancy were not carried out, we cannot be certain.

The observations made in this study make it imperative that an assessment of vitamin D status is made mandatory in Asian women during pregnancy. Women with low 25(OH)D concentrations should be supplemented with vitamin D. Normalisation of calcium and parathyroid hormone concentrations in maternal serum would be useful end points in determining the success of such supplementation and to determine the dose and frequency with which vitamin D preparations are to be administered. In this context, it is worth mentioning that the lower 25(OH)D concentrations in cord blood from Asians are associated with lower calcium and higher PTH concentrations than those from Caucasians.[23] Some Asian neonates in this study had secondary hyperparathyroidism. These infants were born of mothers who had subnormal 25 (OH)D concentrations. Vitamin D supplementation during pregnancy would probably prevent this phenomenon.

The present study demonstrates that even $1,25(OH)_2D$ concentrations are lower in Asian newborns. However, they are comparable to concentrations found in adults. This fact, not reported before, is of interest since four of the newborns had undetectable concentrations of 25(OH)D. Thus, these infants are protected from the effects of vitamin D deficiency by an increased efficiency of 1_α-hydroxylation by the kidney. Whether secondary hyperparathyroidism contributes to an acceleration of 1_α-hydroxylase activity *in utero* is not known, since the placenta, and not the kidney, is the major site for conversion of 25(OH)D to $1,25(OH)_2D$ during pregnancy.[24]

Acknowledgements

The authors are grateful to the nursing staff of the Antenatal Clinic and the Labour Ward for great help and extreme tolerance, to Pamela Dale for preparing the manuscript, and to Drs V Fonseca and D P Mikhailidis for helpful discussion.

References

1 Stanbury SW. Vitamin D and hyperparathyroidism. *J Roy Coll Physicians (London)* 1981; **15:** 205–10.

2 Stephens WP, Klimuik PS, Warrington S *et al.* Observations on the natural history of vitamin D deficiency among Asian immigrants. *Quart J Med* 1982; **52:** 71–80.

3 Dandona P, Mohiuddin J, Weerakoon J *et al.* Persistence of parathyroid hypersecretion after vitamin D treatment in Asian vegetarians. *J Clin Endocrinol Metab* 1984; **59:** 535–7.

4 Cockburn F, Betton NR, Purvis RJ *et al.* Maternal vitamin D intake and mineral metabolism in mothers and their newborn infants. *Br Med J* 1980; **218:** 11–13.

5 Serenius F, Elidrissy AHT, Dandona P. Vitamin D nutrition in pregnant women at term and in newly born babies in Saudi Arabia. *J Clin Pathol* 1984; **37:** 444–7.

6 Okonofua F, Houlder S, Bell J, Dandona P. Vitamin D nutrition in pregnant Nigerian women at term and their newborn infants. *J Clin Pathol* 1986; **39:** 650–3.

7 Reitz RE, Dane TA, Woods JR, Weinstein RL. Calcium, magnesium, phosphorus and parathyroid hormone interrelationships in pregnancy and newborn infants. *Obsts Gynaecol* 1977; **50:** 701–5.

8 Pitkin RM, Reynolds WA, Williams GA *et al.* Calcium metabolism in pregnancy: a longitudinal study. *Am J Obstet Gynecol* 1979; **133:** 781–90.

9 Steichen JJ, Tsang RC, Grattan TL *et al.* Vitamin D homeostasis in the perinatal period. *New Eng J Med* 1980; **302:** 315–19.

10 Whitehead MI, Lane G, Young O *et al.* Interrelations of calcium-regulating hormones during normal pregnancy. *Br Med J* 1981; **283:** 10–12.

11 Dent CE, Gupta MM. Plasma 25-hydroxyvitamin D levels during pregnancy in Caucasians and in vegetarian and non-vegetarian Asians. *Lancet* 1975; **ii:** 1057–60.

12 Rosselin G, Assan R, Yalow RS, Berson SA. Purification of ^{131}I-parathyroid hormone with microfine granules of precipitated silica. *Nature* 1966; **212:** 355–58.

13 Preece MA, O'Riordan JLH, Lawson DEM, Kodicek E. A competitive protein binding assay for 25-hydroxycholecalciferol and 25-hydroxyergocalciferol in serum. *Clin Chim Acta* 1974; **54:** 235–42.

14 Lindall AW, Roos B, Cechettin M. Potential clinical usefulness of new glandular and circulating parathyroid peptides illuminated by sequence specific radioimmunoassay. In Alberto A, Ekins R, eds. *Monoclonal Antibodies and Developments in Immunoassay.* Amsterdam: Elsevier North Holland, 1981; 217–29.

15 Roos B, Lindall AW, Aron DC *et al.* Detection and characterisation of small midregion parathyroid hormone fragments in normal and hyperparathyroid glands and sera by immunoextraction and region specific radioimmunoassay. *J Clin Endocrinol Metab* 1981; **53:** 719–21.

16 Dandona P, Menon RK, Shenoy R *et al.* Low 1,25 dihyroxyvitamin D, secondary hyperparathyroidism and normal osteocalcin in elderly subjects. *J Clin Endocrinol Metab* 1986; **63:** 459–62.

17 Reinhardt TA, Horst RL, Orf JW, Hollis BW. A microassay for 1,25 dihydroxyvitamin D not requiring higher performance liquid chromatography: application to clinical studies. *J Clin Endocrinol Metab* 1984; **58:** 91–6.

18 Stevenson JC, Hillyard CJ, MacIntyre I *et al.* A physiological role of calcitonin: protection of the maternal skeleton. *Lancet* 1979; **i:** 769–70.
19 Lewin LG, Papapoulos SE, Hendy GN *et al.* Reversible resistance to the action of parathyroid hormone in human vitamin D deficiency. *Clin Sci* 1982; **62:** 381–7.
20 Dandona P, Freedman DB, Jeremy JY. Hyperphosphataemic rickets: a new variant. *Br Med J* 1983; **287:** 1765.
21 Robinson D, Flynn D, Dandona P. Hyperphosphataemic rickets in an Asian infant. *Br Med J* 1985; **291:** 1318–19.
22 Dandona P, Okonofua F, Clements RV. Osteomalacia presenting as pathological fractures in pregnant Asian women. *Br Med J* 1985; **290:** 837–8.
23 Okonofua F, Houlder S, Thomas M *et al.* Parathyroid hormone and neonatal calcium homeostasis: evidence for secondary hyperparathyroidism in the Asian neonate. *Metabolism* 1986; **35:** 803–6.
24 Gray TK, Lowy W, Lester GE. Vitamin D in pregnancy: the maternal foetal metabolism of vitamin D *Endocr Rev* 1981; **2:** 264.

Accepted for publication 23 July 1986