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Calcium-Regulating Hormones during the Menstrual Cycle*

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ABSTRACT. Calcium metabolism during the menstrual cycle was studied in seven women from whom fasting blood samples were drawn daily or every other day throughout ovulatory cycles. Total calcium (Ca), ionic calcium (Ca^{++}), magnesium (Mg), phosphorus (P), and immunoreactive parathyroid hormone (PTH) and calcitonin (CT) were measured. LH levels were used to date each cycle and progesterone levels were used to confirm ovulation. Plasma estradiol was measured in two of the subjects.

In six subjects with cycle lengths of 27–31 days, PTH levels rose progressively through the follicular phase to a peak at or slightly before the LH surge, then fell progressively through the luteal phase; peak PTH levels were 30–35% above early follicular and late luteal values. CT levels were also highest at midcycle, but the CT pattern was somewhat more variable than that of PTH. Ca^{++} tended to fall until 3–4 days before ovulation and then to increase, while Ca, Mg, and P exhibited no particular pattern. One subject experienced a prolonged (44 day) ovulatory cycle characterized by three distinct PTH peaks, each of which coincided with elevations in plasma estradiol level.

These results represent the first report of menstrual cyclicity in calcium-regulating hormones. The timing suggests an estrogen effect and it is hypothesized that estrogen inhibits PTH-induced bone resorption, lowering serum Ca^{++}, which in turn provokes a compensatory PTH output. With the decline of the preovulatory estrogen peak, Ca^{++} levels rise and PTH secretion falls. Alternatively, it is possible that the primary action may be an estrogen-induced rise in CT release, causing hypocalcemia and consequent PTH output. Cyclic changes in PRL release or vitamin D metabolism might also be involved. (*J Clin Endocrinol Metab* **47**: 626, 1978)

CALCIUM metabolism consists essentially of a dynamic equilibrium between two calcium pools: a large skeletal pool and a small extracellular fluid pool. A number of homeostatic mechanisms function in concert to regulate this equilibrium in such a manner as to maintain the level of calcium ions in extracellular fluid within narrow physiological limits. The intricate interplay characterizing calcium metabolism is potentially subject to influences by other endocrine mechanisms. We are unaware of any published data regarding calcium metabolism during the menstrual cycle and we here report observations on calcium, other involved electrolytes, and calcium-regulating hormones throughout the menstrual cycle in the human.

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Experimental Subjects

Seven adult women, ranging in age from 22–31 yr, were studied throughout one menstrual cycle each. Six were nulligravid and one had had one previous term pregnancy. All considered themselves in good health and none took any medication during the period of study. Informed consent was obtained from each subject, the investigative protocol having been previously reviewed and approved by the University of Iowa Committee on Research Involving Human Subjects.

Blood was drawn from an antecubital vein beginning on the 2nd or 3rd day of the menstrual cycle (counting the onset of menses as day 1) and continuing every day (in three subjects) or every other day (in four subjects) until the onset of the next menses. All blood samples were collected in vacuum tubes (one plain and one containing heparin) at 0800 h after an overnight fast. The subjects were all ambulatory and engaged in their usual activities during the study. Except for fasting over-

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night until each blood sample was drawn, no specific dietary advice was given.

Materials and Methods

Heparinized blood was centrifuged immediately in cold and the plasma was separated and frozen for later measurement of immunoreactive calcitonin (CT) and, in selected instances, estradiol (E_2) and progesterone. Blood collected in plain tubes was allowed to clot at room temperature for 1 h and was then centrifuged. An aliquot was aspirated, maintaining anerobic conditions as previously described (1), for ionic calcium (Ca^{++}) measurement, and a second aliquot was obtained for determination of total calcium (Ca), magnesium (Mg), and phosphorus (P) concentrations. The remaining serum was then frozen for later assay of immunoreactive parathyroid hormone (PTH) and LH.

Ca^{++} activity was measured with a calcium flow-through electrode (Orion model 801), Ca and Mg concentrations were measured by atomic absorption spectrophotometry (Perkin-Elmer, model 303), and P was measured by the colorimetric method of Fiske and SubbaRow (2). Total protein and albumin concentrations were measured by the biuret method and protein electrophoresis, respectively.

Serum PTH was measured by a modification, described in detail elsewhere (3), of the RIA method of Arnaud *et al.* (4), utilizing guinea pig antibovine PTH antiserum, purified bovine PTH (Wilson Laboratories, lot 147865) tracer, and dilutions of a pool of human parathyroid tissue culture medium for standards. The antiserum detects both carboxyl- and amino-terminals of bovine PTH, has a high affinity for human PTH, and quantitates human PTH appropriately over a 60-fold dilution span. Serum from parathyroidectomized subjects causes no displacement of antibody-bound tracer, whereas normal subjects respond with appropriate serum PTH changes during induced hypo- and hypercalcemia, indicating specificity of the assay. Intra- and interassay coefficients of variation of 2–6% and 3–8%, respectively, have been found. The assay detects as little as 0.5 μl equivalents (μlEq) standard human parathyroid tissue culture medium/ml serum and its normal basal range in humans (mean ± 2 SD) is 3.76–8.56 μlEq/ml.

Plasma CT was measured by a slight modification, decribed in detail elsewhere (5), of the method of Sizemore *et al.* (6), using goat antihuman synthetic CT antiserum and human synthetic CT (N. V. Organon, batch SC 30) for tracer and standards. This antiserum quantitates human CT appropriately over a 60-fold dilution span. No displacement of antibody-bound tracer occurs with either plasma from thyroidectomized subjects or addition of polypeptide hormones other than CT. Appropriate changes in CT levels follow induced hyper- and hypocalcemia. Intra- and interassay coefficients of variation of 1.5–4.5% and 2–7%, respectively, have been found. The assay detects as little as 5 pg CT/ml plasma and its normal basal range (mean ± 2 SD) is 55–380 pg/ml.

The assays for both PTH and CT involve measurement at three dilutions of each serum or plasma sample, with results accepted only if values calculated from the dilutions agree within 10% and differ significantly from the zero point of the standard curve.

LH was measured in each serum sample by RIA (7), with results expressed in relation to LER-907 reference preparation (National Pituitary Agency), and data were tabulated with respect to the maximum LH value in each cycle. Progesterone was measured by a competitive protein-binding technique (8) in three or more plasma samples selected from the last 10 days of each cycle. In two subjects (F and G), E_2 levels were measured by RIA (9) in all samples in which sufficient plasma remained after other determinations.

To examine for possible cross-reactivity between calcium-regulating hormones and gonadotropins, FSH and LH (both in the form of LER-907 standards) were added to seven samples of pooled serum in amounts to yield concentrations ranging from 0–2000 ng LER 907/ml each. Each sample was then assayed for PTH and CT as an unknown.

All determinations were done in duplicate. To obviate interassay variability, assays of the individual hormones (PTH, CT, LH, E_2, and progesterone) from a particular subject were performed in a single assay run.

Results

The length of the menstrual cycle ranged from 27–31 days in six subjects, designated A through F. One (subject G) experienced a prolonged cycle of 44 days. Each cycle studied was ovulatory, as evidenced by a distinct LH peak and a plasma progesterone level ≥8 ng/ml 6–8 days later (8).

Findings in the six subjects with cycle lengths of 27–31 days are summarized in Figs. 1 and 2 and in Table 1. PTH exhibited a cyclic pattern in each subject, with levels generally rising during the follicular phase to a maximum occurring at or slightly before the LH

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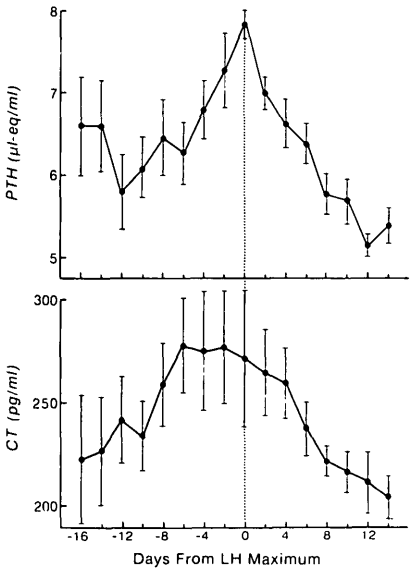

FIG. 1. Mean (±SE) levels of PTH and CT in six subjects during menstrual cycles of 27–31 days.

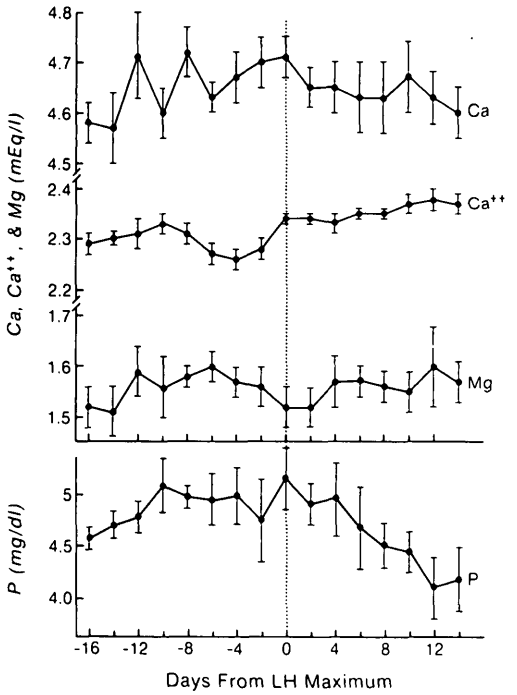

FIG. 2. Mean (±SE) serum levels of Ca, Ca^{++}, Mg, and P in six subjects during menstrual cycles of 27–31 days.

maximum and then falling during the luteal phase. In individual subjects, the PTH peak coincided exactly with the LH surge in four and preceeded it (by 1 and 2 days) in two. Maximal PTH levels averaged 32% (±7 SE) above those of the first 3 days and 35% (±8 SE) above those of the last 3 days of each cycle. CT also tended to rise during the follicular phase to a midcycle maximum and then to fall during the luteal phase. However, CT patterns were less distinct and consistent from subject to subject, as reflected in the relatively wider SEs (Fig. 1). Among individual subjects, the CT maximum coincided with the LH maximum in two, preceeded it (by 2 and 4 days) in two, and followed it (by 1 and 3 days) in two. Maximal CT levels averaged 37% (±17 SE) above those of the first 3 days and 37% (±15 SE) above those of the last 3 days of each cycle.

PTH and CT levels were analyzed statistically by dividing the menstrual cycle into thirds and comparing the middle third (considered as the period from 4 days before to 4 days after the LH surge) to the first and last thirds by two-tailed t test. By this approach in these six subjects, PTH levels during the middle third of the cycle were significantly greater than those of the first ($t = 3.37$, $P < 0.01$) and last ($t = 4.17$, $P < 0.01$) thirds. CT levels during the middle third of the cycle were significantly greater than those of the first ($t = 2.62$, $P < 0.05$) and last ($t = 7.62$, $P < 0.001$) thirds. These data were also anlayzed by two-way analysis of variance, followed by Tukey's multiple comparison test with similar results; *i.e.* midcycle values were significantly greater than first and last thirds in the case of both PTH ($P < 0.005$) and CT ($P < 0.05$).

As illustrated in Fig. 2, Ca and Mg levels varied relatively widely and followed no particular patterns during the menstrual cycle. Ca^{++}, on the other hand, exhibited lesser variation at each point and appeared to decline during the follicular phase and then rise just before ovulation. Among individual subjects,

TABLE 1. Correlation coefficients in subjects with 27- to 31-day cycle lengths

Subject	PTH-Ca	PTH-Ca^{++}	PTH-CT
A	−0.57[a]	−0.52[a]	0.55[a]
B	0.30	−0.71[b]	0.67[b]
C	−0.04	−0.35	0.75[b]
D	0.43	−0.17	0.25
E	−0.23	−0.45[a]	0.71[b]
F	0.35	−0.37[a]	0.18

[a] $P < 0.05$.
[b] $P < 0.01$.

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the Ca^{++} nadir preceded the LH maximum by 4 days in three subjects, by 3 days in one, and by 2 days in two. P levels exhibited considerable variation and, while mean values tended to decline from a midcycle peak, the differences between the middle third of each cycle and either the first or last thirds lacked statistical significance ($P > 0.05$ in each case).

Serum albumin concentration, measured in daily samples from two subjects (E and F), averaged 4.56 (±0.07) and 4.65 (±0.11) g/dl, respectively, and exhibited no pattern in relation to the menstrual cycle. Further, there were no statistically significant correlations between serum albumin and any of the electrolytes or hormones studied ($P > 0.05$ in all cases).

In one of the six subjects with cycle lengths of 27–31 days (subject F), plasma E_2 levels were measured in 21 of 27 daily samples. The follicular phase in this subject was characterized by a typical E_2 peak (296 pg/ml on day −1), falling Ca^{++} levels (to a nadir of 2.18 meq/liter on days −4 and −3), and rising PTH values (to a maximum of 7.83 μlEq/ml on day 0). The luteal phase exhibited secondary E_2 elevations (to 235–240 pg/ml on days 7 and 11) and generally falling PTH values. Ca^{++} concentrations varied during the luteal phase but were higher than those of the follicular phase.

The prolonged (44 day) cycle experienced by subject G differed in some ways from those of the other patients and is considered separately. The cycle was ovulatory, as evidenced by an LH peak of 1497 ng LER 907/ml on cycle day 28 and a plasma progesterone concentration of 11.3 ng/ml 8 days later. However, as illustrated in Fig. 3, PTH exhibited three distinct peaks (on days −16, −2, and +11 from the LH maximum). Statistical analysis of each of the three peaks was done by comparing four measurements about each peak with the four preceding and the four following determinations by two-tailed t test. Statistically significant elevations in PTH were found in all instances ($P < 0.05$ or better). The first PTH peak seemed to be accompanied by elevation of CT, the second less clearly so, and the third not at all. Measurements of E_2 levels,

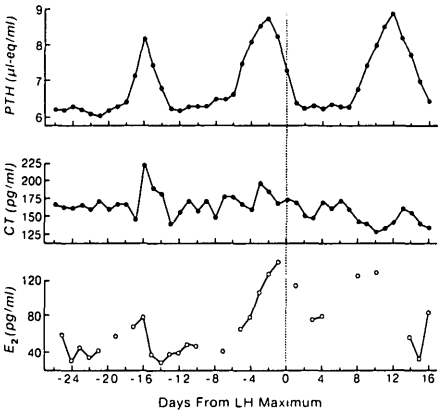

FIG. 3. PTH, CT, and E_2 levels in subjects G during a 44-day menstrual cycle.

possible in only two-thirds of the plasma samples from this cycle, suggested a cyclic pattern similar to that of PTH. An early increment and then decrement in E_2, suggesting partial follicular maturation without ovulation, was accompanied by a PTH rise. Later and more marked E_2 output immediately before ovulation coincided with a higher PTH peak. Finally, a third PTH rise occurred 7–11 days after ovulation in conjunction with an apparent luteal phase elevation of E_2, though unfortunately E_2 values during this time were obtained relatively infrequently.

Addition of LER-907 to pooled serum samples in amounts yielding final concentrations of FSH and LH ranging from 0–2000 ng/ml were assayed at essentially identical concentrations of calcium-regulating hormones. The coefficients of variation were 3.8% and 2.7% in the case of PTH and CT, respectively, indicating a lack of cross-reactivity between gonadotrophic and calcium-regulating hormones.

Discussion

This report represents the first systematic study of calcium metabolism during the menstrual cycle and demonstrates a remarkable cyclicity in levels of the calcium-regulating hormones, PTH and CT. The PTH pattern was particularly consistent and distinctive, consisting of a rise during the follicular phase of the cycle to a maximum at or slightly before ovulation, followed by a progressive decrement during the luteal phases. Early follicular

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and late luteal values tended to lie in the upper portion of the normal range for our assay and peak values were at, or in some instances slightly above, the upper limit of normal.

The explanation of these alterations in PTH levels cannot be stated with certainty. However, the temporal relationships suggest an association with endogenous estrogen secretion. Considerable evidence from a number of sources indicates that estrogens influence calcium metabolism. Serum calcium levels typically rise with cessation of menstrual function (10), a time well known to be associated with an increased propensity to osteoporosis in the female (11). Treatment with natural and/or synthetic estrogen lowers the serum calcium level in both premenopausal (12) and postmenopausal (13–15) women, as well as retarding development of osteoporosis in the latter group (16). Further evidence of the calcium-lowering action of estrogen comes from observations that the clinical onset of primary hyperparathyroidism seems particularly likely with menopause (17) and that estrogen treatment of postmenopausal hyperparathyroid patients lowers serum and urinary calcium levels (18). Further, estrogen administration has been reported to precipitate hypocalcemic tetany in a patient with hypoparathyroidism (19).

The mechanism by which estrogen lowers serum calcium is generally regarded to be through an inhibition of bone resorption. *In vitro* studies have demonstrated that natural and synthetic estrogens inhibit PTH-induced calcium release from organ culture of mouse calvaria (20). Castration has been reported to increase bone sensitivity to PTH in rats studied *in vivo* (21). Estrogen treatment in humans has been noted to promote increased bone mineralization (22) and both endogenous (23) and exogenous (13–15, 24) estrogen to lower urinary excretion of calcium and hydroxyproline, effects reflecting a lowered resorption rate. Heany (25) has pointed out that estrogen apparently inhibits bone resorption by suppressing the mesenchymal process involved in bone remodeling, while PTH acts in an exactly opposite manner. Thus, in a general way, estrogen and PTH seem to have antagonistic actions on bone metabolism. If estrogen lowers serum Ca^{++} levels by decreasing bone responsiveness to PTH, a compensatory increase in PTH secretion would be a reasonable expectation. Confirmatory of this concept is the study of Riggs *et al.* (26), who found a significant increase in PTH levels with administration of conjugated estrogens to women with postmenopausal osteoporosis.

With this background, it may be speculated that the findings of the present study reflect a course of events initiated by increasing estrogen secretion as the Graffian follicle matures. Inhibition of bone resorption by these increasing estrogen levels results in falling serum Ca^{++} levels, calling forth a compensatory PTH output and thereby preventing marked degrees of hypocalcemia. Estrogen output reaches a maximum immediately before ovulation and its subsequent decline leads to a rise in Ca^{++} and concomitant fall in PTH. This proposed explanation does not account for the progressive PTH decrement during the luteal phase, when estrogen characteristically exhibits a secondary rise. Indeed, our subject G displayed a marked luteal phase PTH elevation as well as a smaller one during the prolonged follicular phase, and each appeared to coincide with an increase in E_2 levels (Fig. 3). In addition, one of the other subjects (subject F) had a slight secondary rise in PTH levels, maximal on the 6th postovulatory day. However, the fact remains that the majority exhibited a progressive decrement in circulating PTH levels after a maximum at or slightly before ovulation. Progesterone may be involved and if so might exert effects on bone metabolism opposite to that of estrogen, just as it does in a variety of other organs. Influences of progesterone *per se* on calcium metabolism have been studied little, although *in vitro* observations suggest that this hormone inhibits bone resorption in a manner similar to that of estrogens (27).

CT levels tended to parallel those of PTH in the present study, although the pattern of CT seemed more variable and the midcycle elevation seemed considerably less distinct than was the case with PTH. The explanation

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of the observed changes in CT is obscure. Falling Ca^{++} levels during the follicular phase implies that CT should, if anything, decline. However, the negative feedback system governing CT secretion may not be under the same fine degree of control as is that of PTH. Nevertheless, why PTH and CT should follow generally parallel courses is unclear. Other studies (28) have indicated, however, that levels of the two calcium-regulating hormones tend to be positively correlated under basal conditions in normal subjects as well as in some with chronic renal failure.

In addition to the hypothesis that estrogen-induced lowering of the serum calcium level represents the primary cause of the observations noted in this study, it is also conceivable that the primary action involves stimulation of CT secretion. In this regard, Klotz *et al.* (29) have reported that estrogen treatment of castrated rats increases levels of bioassayable CT activity. Thus, an alternative explanation of our findings would be an estrogen-induced rise in CT, producing hypocalcemia and thus eliciting a compensatory PTH output.

Cyclic variation in reproductive hormones other than estrogen could conceivably play a role in calcium metabolism. PRL levels are maximal at ovulation (30) and if this hormone shares the hypercalciuric action of GH, such an effect might lead to the changes observed.

Vitamin D, another humoral substance regulating calcium metabolism, might also be involved. Although studies of vitamin D and its metabolites have not been reported in relation to the menstrual cycle or hormonal treatment, serum levels of 25-hydroxyvitamin D_3, the principal circulating form of the vitamin, do not seem to be affected remarkably by pregnancy (31, 32). Of possible significance is a recent study (33) in the Japanese quail describing marked increases in renal conversion of 25-hydroxyvitamin D_3 to its more active 1,25-dihydroxy form at about the time of ovulation. If a similar event occurs in mammals, it might represent an effect of increased PTH secetion.

Alterations in calcium metabolism with pregnancy are of interest in relation to the present study because of certain similarities between gestational and menstrual physiology. Elevated PTH levels (34) and loss of trabecular bone (35) have been found in normal pregnancy, events which could conceivably reflect hyperestrogenism. However, pregnancy is accompanied by numerous other factors with known or potential influences on calcium metabolism, such as the obligatory transplacental calcium loss, increased renal excretion, hypoalbuminemia, and placental lactogen elaboration, making any analogy between the present data and pregnancy effects tenuous at best.

Finally, it should be acknowledged that blood levels of any hormone cannot be regarded categorically as reflecting changes in secretion or production, as levels depend on the metabolic clearance rate as well as the rate of production. However, alterations in metabolic clearance during the normal menstrual cycle to an extent sufficient to account for the changes in hormone levels observed in this study do not seem likely. Therefore, it seems reasonable to regard the patterns of PTH and CT concentrations reported here as reflecting cyclic variation in production of these calcium-regulating hormones.

From the clinical point of view, this demonstration of menstrual cyclicity of calcium-regulating hormone levels indicates that the stage of the cycle should be taken into account when these assays are used for diagnostic purposes in women of reproductive age.

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References

1. PITKIN, R. M., AND M. P. GEBHARDT, Serum calcium concentrations in human pregnancy, *Am J Obstet Gynecol* **127**: 775, 1977.
2. NATELSON, S., Microtechniques of Clinical Chemistry, ed. 2, Springfield, Charles C Thomas, 1963, p. 335.
3. HARGIS, G. K., G. A. WILLIAMS, W. A. REYNOLDS, W. KAWAHARA, B. JACKSON, E. N. BOWSER, AND R. M. PITKIN, Radioimmunoassay of parathyroid hormone in monkey and man, *Clin Chem* **23**: 1989, 1977.
4. ARNAUD, C. D., H. S. TESO, AND T. LITTLEDIKE, Radioimmunoassay of human parathyroid hormone in serum, *J Clin Invest* **50**: 21, 1972.
5. HARGIS, G. K., W. A. REYNOLDS, G. A. WILLIAMS, W. KA-

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WAHARA, B. JACKSON, E. N. BOWSER AND R. M. PITKIN, Radioimmunoassay of calcitonin in the plasma of rhesus monkey and man, *Clin Chem,* **24:** 595, 1978.
6. SIZEMORE, G. W., V. L. W. GO, E. L. KAPLAN, L. J. SANZENBACHER, K. H. HOLTENMULLER, AND C. D. ARNAUD, Relations of calcitonin and gastrin in the Zollinger-Ellison syndrome and medullary carcinoma of the thyroid, *N Engl J Med* **288:** 641, 1973.
7. ODELL, W. D., G. T. ROSS, AND P. L. RAYFORD, Radioimmunoassay of luteinizing hormone in human plasma or serum: physiological studies, *J Clin Invest* **46:** 248, 1967.
8. ISRAEL, R., D. R. MISHELL, S. C. STONE, I. H. THORNEYCROFT, AND D. L. MOYER, Single luteal phase serum progesterone assay as an indicator of ovulation, *Am J Obstet Gynecol* **112:** 1043, 1972.
9. KORENMAN, S. G., R. H. STEVENS, L. A. CARPENTER, M. ROBB, G. D. NISWENDER, AND B. M. SHERMAN, Estradiol radioimmunoassay without chromatography: procedure, validation and normal values, *J Clin Endocrinol Metab* **38:** 718, 1974.
10. YOUNG, M. N., AND B. E. C. NORDIN, Effects of natural and artificial menopause on plasma and urinary calcium and phosphorus, *Lancet* **2:** 118, 1967.
11. HEANY, R. P., A unified concept of osteoporosis, *Am J Med* **39:** 877, 1965.
12. SIMPSON, G. R., AND E. DALE, Serum levels of phosphorus, magnesium and calcium in women utilizing combination oral or long-acting injectable progestational contraceptives, *Fertil Steril* **23:** 326, 1972.
13. YOUNG, M. N., C. JANARI, D. A. SMITH, AND B. E. C. NORDIN, Some effects of ethinyloestradiol on calcium and phosphorus metabolism in osteoporosis, *Clin Sci* **34:** 411, 1968.
14. GALLAGHER, J. C., AND B. E. C. NORDIN, Effects of estrogen and progestagen therapy on calcium metabolism in postmenopausal women, *Front Horm Res* **3:** 150, 1975.
15. AITKEN, J. M., D. M. HART, AND D. A. SMITH, The effect of long-term mestranol administration on calcium and phosphorus homeostasis in oophorectomized women, *Clin Sci* **41:** 233, 1971.
16. AITKEN, J. M., D. M. HART, AND R. LINDSAY, Oestrogen replacement therapy for prevention of osteoporosis after oophorectomy, *Br Med J* **3:** 515, 1973.
17. PYRAH, L. N., A. HODGKINSON, AND C. K. ANDERSON, Primary hyperparathyroidism. Critical review, *Br J Surg* **53:** 245, 1977.
18. GALLAGHER, J. C., AND R. WILKINSON, The effect of ethinyloestradiol on calcium and phosphorus metabolism of postmenopausal women with primary hyperparathyroidism, *Clin Sci Mol Med* **45:** 785, 1973.
19. BURNKHARDT, P., B. RUEDI, AND J. P. FELBER, Estrogen-induced tetany in idiopathic hypoparathyroidism, *Horm Res* **6:** 321, 1975.
20. ATKINS, D., J. M. ZANELLI, M. PEACOCK, AND B. E. C. NORDIN, The effect of oestrogens on the response of bone to parathyroid hormone *in vitro, J Endocrinol* **54:** 107, 1972.
21. ORIMO, H., T. FUJITA, AND M. YOSHIKAWA, Increased sensitivity of bone to parathyroid hormone in ovariectomized rats, *Endocrinology* **90,** 760, 1972.
22. GOLDSMITH, N. F., AND J. O. JOHNSTON, Bone mineral: effects of oral contraceptives, pregnancy, and lactation, *J Bone Joint Surg* **57:** 657, 1975.
23. LINDSAY, R., J. R. T. CULTS, AND D. M. HART, The effect of endogenous oestrogen on plasma and urinary calcium and phosphate in oophorectomized women, *Clin Endocrinol* **6:** 87, 1977.
24. GOULDING, A., AND R. MCCHESNEY, Oestrogen-progestogen oral contraceptives and urinary calcium excretion, *Clin Endocrinol* **6:** 449, 1977.
25. HEANY, R. P., Estrogens and postmenopausal osteoporosis, *Clin Obstet Gynecol* **19:** 791, 1976.
26. RIGGS, B. L., J. JOWSEY, R. S. GOLDSMITH, P. J. KELLY, D. L. HOFFMAN, AND C. D. ARNAUD, Short- and long-term effects of estrogen and synthetic anabolic hormone in postmenopausal osteoporosis, *J Clin Invest* **51:** 1659, 1972.
27. ATKINS, D., AND M. PEACOCK, A comparison of the effects of the calcitonins, steroid hormones, and thyroid hormones on the response of bone to parathyroid hormone in tissue culture, *J Endocrinol* **64:** 573, 1975.
28. HEYNEN, G., J. A. KANIS, D. OLLIVER, J. G. G. LEDINGHAM, AND R. G. G. RUSSELL, Evidence that endogenous calcitonin protects against renal bone disease, *Lancet* **2:** 1322, 1976.
29. KLOTZ, H.-P., M.-L. DELORME, F. OCHOA, AND C. AUSSENARD, Hormones sexuelles et secretion de calcitonine, *Sem Hop Paris* **51:** 1333, 1975.
30. VEKEMANS, M., P., DELVOYE, M. L'HERMITE, AND C. ROBYN, Serum prolactin levels during the menstrual cycle, *J Clin Endocrinol Metab* **44:** 989, 1977.
31. DENT, C. E., AND M. M. GUPTA, Plasma 25-hydroxyvitamin D levels during pregnancy in Caucasians and in vegetarian and non-vegetarian Asians, *Lancet* **2:** 1057, 1975.
32. TURTON, C. W. G., T. C. B. STAMP, P. STANLEY, AND J. D. MAXWELL, Altered vitamin D metabolism in pregnancy, *Lancet* **1:** 222, 1977.
33. KENNY, A. D., Vitamin D metabolism: physiological regulation in egg-laying Japanese quail, *Am J Physiol* **230:** 1609, 1976.
34. CUSHARD, W. G., S. CREDITOR, J. M. CANTERBURY, AND E. REISS, Physiologic hyperparathyroidism in pregnancy, *J Clin Endocrinol Metab* **34:** 767, 1972.
35. LAMKE, B., J. BRUNDIN, AND P. MOBERG, Changes of bone mineral content during pregnancy and lactation, *Acta Obstet Gynecol Scand* **56:** 217, 1977.

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