SERUM PHOSPHORUS DURING
THE MENSTRUAL CYCLE

by
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APPROVAL BY THESIS DIRECTOR

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July 9, 1979
This thesis is lovingly dedicated to my husband, Kim, who has given me continued love, support, encouragement, and understanding. His unending patience during this difficult time will never be forgotten. I love you Kim.
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ABSTRACT

Serum phosphorus was measured in nine ovulatory and two anovulatory women during one menstrual cycle. A descriptive design was used to determine whether there was a significant periovulatory period increase in serum phosphorus in ovulating women. Indirect indicators of ovulation were the biphasic basal body temperature curve and luteal phase progesterone levels of 3 ng/ml or more. No significant periovulatory period change in serum phosphorus was found. Six of nine ovulating women showed a slight nonsignificant decrease in serum phosphorus at the estimated time of ovulation followed by a luteal phase increase. Conversely, two anovulatory women showed a nonsignificant periovulatory period increase in serum phosphorus which preceded the progesterone rise. The absence of a periovulatory period serum phosphorus peak in this study versus the salivary peak found by an earlier study suggests that there may be altered phosphorus metabolism around ovulation and that salivary glands, via hormonal control, may function to maintain serum phosphorus within normal limits.
CHAPTER 1
INTRODUCTION

Birth control is a topic which concerns many people. Whether one's perspective is limited to the personal effects on individuals and families of high fertility or the total effects on nations and the world, improving the regulation of fertility and the resulting reduction in fertility levels are urgent objectives in industrial as well as developing nations (Creep, Koblinsky, and Jaffe 1976). Mushrooming population growth, if not controlled, will have devastating effects on our environment, the economic development of the world and indeed the quality of life itself.

The annual rate of world population growth is presently two percent, a rate without precedent in history (Greep et al. 1976). The rate of population growth was almost zero from the time of the appearance of humankind on earth up to early historical times. The average annual rate of growth had increased to about 0.3 percent by 1700's, reaching one percent in the 1950's and then only 20 years later, the annual rate of population growth had doubled to two percent (Greep et al. 1976). Greep et al. note that if this rate of two percent population growth continues, then in just 300 years the entire land area of the earth would have the density of New York City today; in 600 years there would be one person per square meter of land area; and finally in
1200 years the weight of the world population would be greater than the mass of the earth (Creep et al. 1976).

Multiple methods of birth control are being offered to the public today. However, many of these methods have questionable effectiveness while others have questionable safety. For example, "the pill" and the intrauterine device (IUD) are designed to interrupt normal female reproductive processes. How these products function is unknown. Yet millions of women around the world use these products for contraception, and they do so at the risk of death or developing a chronic disease from pill or IUD use. In the Sixth Annual Report by the World Health Organization the present status of family planning is made explicit: "Current technology has limitations with respect to efficacy, safety, acceptability and continuity of use which result in unacceptably high failure rates and require large-scale resort to abortion as a back-up measure . . ." (World Health Organization 1977, p. 5). The safety issue is significant enough to make the most effective methods of birth control available today unacceptable to some women and induce enough concern in others to militate against regular use. WHO concluded in this report that current technology cannot be considered adequate in either industrial or developing nations to meet individual or societal needs (WHO 1977).

Thus the search goes on for a simple, painless, rapid, reliable, specific and inexpensive method of birth control. The only completely safe and effective method of fertility control available today, in spite of the increasing knowledge in the biological sciences, is sexual abstinence. Authors note that periodic abstinence is practical when one
considers the proposed short fertile half life of both ova and sperma-
zoa (Rosado et al. 1977). Therefore, in addition to the search for
an inexpensive, simple and reliable method of birth control, a search
is simultaneously proceeding for methods which predict when ovulation
will or has occurred. If an indirect indicator of ovulation could be
found, a reliable and efficient way of identifying the peri-fertile
period; sexual abstinence during this peri-fertile period would prevent
fertilization. This approach to birth control, if developed, has the
potential of meeting the above criteria: simple, painless, rapid, re-
liable, specific, inexpensive and thus extremely desirable.

A variety of substances have been found to fluctuate during the
periovulatory period of the menstrual cycle. It is postulated that one
of these substances may have the potential to be an indirect indicator
of ovulation. Most recently Ben-Aryeh et al. (1976) found a signifi-
cant periovulatory period increase in salivary phosphate, while Pitkin
et al. (1978) found that serum phosphorus exhibited considerable varia-
tion but with no significant or particular periovulatory period pattern.
Other substances examined have been: cervical mucous pattern (Billings
and Billings 1972), salivary glucose (Davis et al. 1974), alkaline phos-
phatase, arylsulfatase and B-Glucuronidase in saliva (Boyer and France
1976), nucleic acid and electrolyte content in human endometrium
(Liedtke, Neukirchen, and Lang 1975), N-acetyl-B-D glucosaminadase
(Rosado et al. 1977).
Statement of the Problem

The problems of this thesis were to determine whether serum phosphorus levels fluctuate during the menstrual cycle in ovulating women; and specifically, was there a significant periovulatory period increase in serum phosphorus in ovulating women.

Purpose of the Study

The purpose of this study was to measure serum phosphorus in ovulating women during one menstrual cycle to see if there was a significant periovulatory period increase in phosphorus. Identification of whether serum phosphorus peaks during the periovulatory period in ovulating women will add to the body of scientific knowledge of the menstrual cycle and contribute to the search for a safe, effective indirect indicator of ovulation.

Significance of the Study

The search for a safe and effective method of birth control is a goal shared by many of the health sciences, including the science of nursing. The findings of this study, however, are relevant to nursing not only due to the information provided regarding birth control; there are nutritional implications as well. Regulation of the essential body elements, calcium and phosphorus is influenced by the hormonal interactions surrounding the menstrual cycle (Pitken et al. 1978). The dietary requirements of the body for calcium and phosphorus will be affected by these hormonal interactions. Describing the relationships between calcium, phosphorus and the menstrual cycle will provide
information for the nurse teacher enabling him/her to better counsel women regarding their nutritional needs.

**Definition of Terms**

The *periovulatory period* is defined as two days prior to ovulation, day of ovulation, and two days after ovulation.
CHAPTER 2

SELECTED REVIEW OF LITERATURE

A variety of endogenous biochemical substances have been found to fluctuate during the periovulatory period of the menstrual cycle: salivary glucose, alkaline phosphatase, arylsulfatase, B-Glucuronidase, N-acetyl-B-D-glucosaminidase, and of particular interest to this study, phosphorus (Davis et al. 1974; Ben-Aryeh et al. 1976; Boyer and France 1976; Rosado et al. 1977). One possible explanation for the periovulatory period changes detected by some investigators of these substances are the mechanisms involved in the release of the hypothalamic gonadotrophic releasing hormones and the pituitary gonadotropins themselves. These mechanisms are all energy demanding and consuming, requiring a variety of inorganic catalysts such as magnesium. Substances that have been found to fluctuate in parallel with the periovulatory period may be cofactors or byproducts of the reactions occurring around ovulation. These mechanisms may be intimately associated with phosphorus.

This chapter includes a discussion of regulation of the menstrual cycle; a review of phosphorus to include uses of phosphorus in the body, phosphorus metabolism and regulation, and what is known about phosphorus's relationship to the menstrual cycle.
Regulation of the Menstrual Cycle

The human reproductive process, on which the size and structure of individual families and of the populations of communities, nations and the world ultimately depend, is an orchestration, of interrelated behavioral and physiological events and anatomical changes that proceed in perfect sequence and synchrony (Segal 1974, p. 53).

The events which proceed in perfect sequence and synchrony are regulated by a series of chemical substances that issue from the brain and the pituitary gland to influence the gonads and then from the sex glands to order the successive events of egg or sperm development and transport, fertilization, implantation and gestation (Segal 1974).

The central nervous system is known to play a major role in regulating reproductive function in women (Naftolin and Tolis 1978; Segal 1974). The observation has been made that ovulation can be provoked or prevented by either electrically stimulating or making anatomic lesions of the endocrine hypothalamus (Naftolin and Tolis 1978). Studies also indicate that extrahypothalamic structures such as the limbic system are connected with the hypothalamic area both functionally and anatomically, and in animals are involved in the mechanism of ovulation (Naftolin and Tolis 1978).

When the hypothalamus is stimulated by the appropriate molecular message a stored supply of gonadotropin releasing hormone, a decapeptide, is secreted. This releasing hormone is a glycoprotein called luteinizing hormone releasing hormone (LHRH). LHRH travels from the hypothalamic cells to the anterior lobe of the pituitary by way of the hypothalamus-pituitary portal system (Schally, Arimura, and Kastin 1973). LHRH then causes the pituitary to discharge its stored supply of two
large glycoproteins, the gonadotropins called luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH enter the bloodstream and are carried to the reproductive target organs (Schally 1978). The reproductive target organ, the ovary, then synthesizes and secretes the sex hormones, primarily estrogen and progesterone.

The question regarding the existence of more than one gonadotropin-releasing factor remained in dispute for many years. Schally and his collaborators took the bold step in their 1971 publication of proposing that one hypothalamic hormone designated LH-RH/FSH-RH could be responsible for the dual effect of possessing major FSH-RH as well as LH-RH activity. This concept is now supported by many physiological as well as immunological data (Schally 1978).

Many analogs of luteinizing hormone releasing hormone have been synthesized and as a result of their synthesis and research in this area several principles regarding luteinizing hormone releasing hormone have become apparent (Naftolin and Tolis 1978): "Luteinizing hormone releasing hormone acts by binding to pituitary cell membrane's specific binding sites; the action of luteinizing hormone releasing hormone seems to trigger intracellular systems via adenyl cyclase and prostoglandin" (Naftolin and Tolis 1978, p. 21). These authors go on to explain that when the structure of luteinizing hormone releasing hormone analogs are altered one can manipulate the degree of agonist (prorelease) or antagonist (antirelease) activity; possibly due to different cell-membrane-binding and activating characteristics of the analogs or due to the competitive inhibition with endogenous substances (i.e., LHRH) which would bind to these receptor sites (Naftolin and Tolis 1978). These
analogs are being used for specific diagnostic and therapeutic purposes (Naftolin and Tolis 1978).

Much research is being done in the area of luteinizing hormone releasing hormone and its release as well as release of the gonadotropins by the anterior pituitary. Neurotransmitters such as biogenic amines, peptides and steroids act to stabilize and destabilize the membranes of the luteinizing hormone releasing hormone-containing neurons (Naftolin and Tolis 1978). There is also evidence that neurotransmitters may affect release of the gonadotropins from the anterior pituitary directly (Naftolin and Tolis 1978).

In addition to the neurotransmitter effect on control of luteinizing hormone releasing hormone and gonadotropins, the sex steroids are of great importance as well. Sex steroids, estrogens, progesterones, and androgens feedback at the pituitary level as well as to the hypothalamus (Schally 1978).

Feedback

The concept of feedback is an extremely important one in regulation of the menstrual cycle. The reproductive system is a group of biologically discrete units which evolve and interact throughout a woman's lifetime. The mechanisms found in the normal adult woman form a functional unit of reproduction which is made up of subunits or modules. The central neuroendocrine axis, which is made up of the endocrine brain and the pituitary, the gonads and the adrenal glands, and the internal and external genitalia are the most important of these modules. The term feedback describes the relationship between the central neuroendocrine
axis and the peripheral endocrine production. The key to a properly functioning functional unit of reproduction is the development and maintenance of proper feedback (Naftolin and Tolis 1978).

Both negative and positive feedback mechanisms operate to regulate the menstrual cycle (Guyton 1976; Naftolin and Tolis 1978; Speroff and Vande Wiele 1971; Schally 1978).

**Negative Feedback.** Negative feedback occurs when increased levels of estrogen inhibit the production of FSH and LH (Speroff and Vande Wiele 1971).

**Positive Feedback.** The positive feedback effect of estrogen is demonstrated by the LH surge that must occur prior to ovulation. As estrogen levels increase, they reach a critical concentration approximately 24 hours prior to ovulation. At this time a dramatic increase in LH occurs (Schally 1978). This positive feedback effect of estrogen is on luteinizing hormone secretion but does not appear to affect follicle-stimulating hormone secretion (Speroff and Vande Wiele 1971). Guyton discusses experiments in which estrogen is infused in a woman for a period of two-three days during the first half of the ovarian cycle and cause rapidly accelerating growth of the follicles and rapidly accelerating secretion of ovarian estrogens (Guyton 1976). At first follicle-stimulating hormone and luteinizing secretion by the anterior pituitary is suppressed (negative feedback), but then abruptly the secretion of luteinizing hormone increases about seven fold and the secretion of follicle-stimulating hormone about two fold (Guyton 1976). This is thought to be caused from the positive feedback of estrogen which overcomes the negative feedback that occurs in the remainder of
the menstrual cycle (Guyton 1976). Naftolin and Tolis (1978) speculate that this positive feedback may involve increased secretion from the hypothalamus of luteinizing hormone releasing hormone and/or an increased sensitivity of the pituitary gonadotropes to luteinizing hormone releasing hormone which is translated as an enhanced response per unit of luteinizing hormone releasing hormone received. Both sex steroids and luteinizing hormone releasing hormone appear to be important in pituitary sensitization (Naftolin and Tolis 1978). The exact roles of increased luteinizing hormone releasing hormone secretion as opposed to increased pituitary sensitization to luteinizing hormone releasing hormone in the positive feedback mechanism is not clear. Schally supports the view of increased pituitary sensitization to LHRH in the positive feedback mechanism as well as large amounts of estrogen and progesterone which are secreted after ovulation lowering pituitary responsiveness to LHRH, i.e., negative feedback (Schally 1978). The exact cause of the LH surge is not known. It is known that the LH surge leads to ovulation with subsequent formation of the corpus luteum and the cycle begins again.

As noted earlier, a variety of endogenous biochemical substances have been found to fluctuate during the periovulatory period of the menstrual cycle: salivary glucose, alkaline phosphatase, arylsulfatase, B-Glucuronidase, N-Acetyl-B-D-glucosaminidase, and of particular interest to this study, phosphorus (Ben-Aryeh et al. 1976; Boyer and France 1976; Davis et al. 1974; Rosado et al. 1977). One possible explanation for the periovulatory period changes detected by some investigators of these substances are the mechanism involved in the release of the hypothalamic
gonadotrophic releasing hormones and the pituitary gonadotropins themselves. These mechanisms are all energy demanding consuming, requiring a variety of inorganic catalysts, such as magnesium. Substances that have been found to fluctuate in parallel with the periovulatory period may be cofactors or byproducts of the reactions occurring around ovulation. These mechanisms may be intimately associated with phosphorus, specifically the catalytic conversion of adenosine triphosphate (ATP) to cyclic-3'5'-adenosine monophosphate (cAMP). To illustrate, LH, acting as "first messenger," travels to the target cell, the ovary, and binds to specific receptor sites on the outside of the cell wall. The activity of the adenylate cyclase in the cell membrane is increased with the binding of the hormone to the receptor site. As a result of this activity cyclic AMP is produced utilizing an abundant supply of ATP on the inner side of the cell membrane. Cyclic AMP then acts as a "second messenger" diffusing throughout the cell instructing the cell to respond in a characteristic way (Pastan 1972; Sutherland 1972). In this reaction a phosphorus compound is metabolized with the release of two phosphate molecules in the formation of cAMP. In this way phosphorus is involved with regulation of the menstrual cycle.

**Phosphorus**

Total body stores of phosphorus are estimated at 700-800 grams by Kreisberg (1977); others estimate that it is higher, 1000 grams (Krane and Potts 1977). About 85 percent of total body phosphorus is located in the skeleton; the remaining 100-120 grams is found in soft tissues (Kreisberg 1977). Normal serum phosphorus levels in the fasting state range from 2.8 to 4.0 mg/100ml (Krane and Potts 1977). Most
serum phosphorus is not bound; 12 percent is said to be bound to proteins (Krane and Potts 1977).

Just as potassium is the major intracellular cation, phosphorus is the major intracellular anion with its average concentration in cell water being 100 mmoles/liter (Knochel 1977). Most all intracellular phosphorus is organic in the form of intermediary carbohydrate, lipids, and proteins, with only a small fraction being inorganic (Knochel 1977). The low concentration of inorganic phosphorus is very important in terms of function, it is from this source of phosphorus from which ATP, the metabolic energy material, is resynthesized (Knochel 1977). Inorganic refers to mineral substances or being composed of matter other than plant or animal, substances classified other than organic. Organic is defined as carbon compounds of living beings and most other carbon compounds.

Uses of Phosphorus in the Body

The physiological functions of phosphorus are both varied and important. It is an "integral and critical constituent of all body tissues" (Kreisberg 1977, p. 121). Specifically, phosphorus is essential for the structure and function of all cells and all synthetic as well as catabolic processes (Knochel 1977). Both internal and external cellular membranes, as well as intracellular membranes that form organelles contain phospholipids as their predominant type of lipid. Thus phosphorus plays an essential role in the structural integrity of all body cells (Kreisberg 1977). Also, in terms of structure, phosphorus is the major component of the mineral phase bone; the mineral phase is composed of calcium and phosphorus (Krane and Potts 1977). Bone is
formed by a process in which inorganic mineral is deposited in relation to an organic matrix (Krane and Potts 1977).

Phosphorus is found in nucleic acids as well as phosphoproteins, both important constituents of mitochondria where they function to permit the generation and storage of chemical energy (Kreisberg 1977). "The energy itself is stored in the form of high energy phosphate compounds, notably adenosine triphosphate (ATP) the energy source for a host of physiologic processes, including muscle contraction, electrolyte transport, and a great variety of biosynthetic reactions" (Kreisberg 1977, p. 122).

Another phosphorus containing compound is 2,3-diphosphoglyceric acid (2,3 DPG). This compound primarily regulates the affinity hemoglobin has for oxygen, permitting release of oxygen to the tissues (Kreisberg 1977).

Phosphorus functions to regulate the activities of many enzymes, cofactors, and biochemical intermediates--"notably those involved in the storage and breakdown of glucose become activated only through the addition of a phosphate group" (Kreisberg 1977, p. 122). Enzymes such as glucokinase, hexokinase, fructokinase, and galactokinase are examples of substances dependent upon phosphorus to carry out their functions in the metabolism of carbohydrates (Guyton 1976).

Phosphorus compounds compose an important urinary buffer system which helps keep the pH within physiologic limits. When a strong acid such as hydrochloric acid reacts with the phosphate buffer system the net result is the removal of hydrochloric acid and in its place an additional quantity of a weakly acidic phosphorus compound is formed.
Therefore a strong acid has been traded for a weak acid permitting only a slight change in pH (Guyton 1976, p. 488).

\[
\text{HCl} + \text{Na}_2\text{HPO}_4 \rightarrow \text{NaH}_2\text{PO}_4 + \text{NaCl}.
\]

Conversely if a strong base such as sodium hydroxide is added to the phosphate buffer system a reaction takes place which results in the exchange of a strong base with a weak base allowing for only a slight shift in pH to the alkaline side (Guyton 1976, p. 488).

\[
\text{NaOH} + \text{NaH}_2\text{PO}_4 \rightarrow \text{NaHPO}_4 + \text{H}_2\text{O}.
\]

And finally, phosphorus plays a critical role in the defense against infectious organisms (Knochel, 1977). "ATP is required for the formation of phagocytic vacuoles in white cells and provides energy for membrane work, amoeboid movement, and pseudopod formation. Organophosphorus compounds are building blocks for membrane synthesis and phagocytic granules" (Fitzgerald 1978, p. 183).

**Phosphorus Metabolism and Regulation of Metabolism**

Cyclic 3'-5'-adenosine monophosphate plays a central role in the regulation of metabolism of cells, in addition to its major role in the regulation of the menstrual cycle (Pastan 1972; Sutherland 1972; Tepperman 1973). A serious deficiency of phosphorus could lead to dangerous and perhaps fatal consequences. Fortunately phosphorus deficiency is rare due in part to the fact that the normal diet contains some 800 to 1000 mg of phosphorus per day from such sources as meat, fish, poultry, eggs, milk, cheese, nuts and legumes (Kreisberg 1977). Another factor in preventing phosphorus deficiency is the fact that the kidney is very
efficient in recycling the phosphorus: 85 to 90 percent of the filtered phosphate is reabsorbed by the proximal tubule (Kreisberg 1977).

The absorption of phosphorus from the intestine is also very efficient. "At low levels of dietary intakes (less than 2 mg/kg/day) 80 to 90 percent of ingested phosphorus is absorbed. Even at higher levels of intake (greater than 10 mg/kg/day) usually encountered in average diets in the form of dairy products, cereals, eggs, and meat, absorption is about 70 percent" (Krane and Potts 1977, p. 2008).

Guyton also notes that phosphorus is absorbed exceedingly well; however, he adds if there is excess calcium in the diet, calcium tends to form almost insoluble calcium phosphate compounds that fail to be absorbed and which then pass on through the bowels to be excreted in the feces (Guyton 1976). "The major problem in absorption of calcium and phosphate is actually a problem of calcium absorption alone, for if this is absorbed both are absorbed" (Guyton 1976, p. 1052).

Phosphorus is referred to as a "threshold substance;" if the concentration of phosphate in the plasma is below the critical value of approximately one millimol/liter, no phosphate at all is lost in the urine; but above this critical concentration, the rate of phosphorus loss is directly proportional to the additional increase (Guyton 1976).

Krane and Potts (1977, p. 2008) also agree that "the major control of phosphorus economy is exerted at the level of the kidney." Specifically when filtered loads of phosphorus decrease, the proximal tubular reabsorption increases, and conversely, when phosphorus loads are increased, tubular reabsorption decreases and clearance rises. Therefore, conservation or elimination of excessive amounts of the ion
is normally reflected in the urinary excretion of phosphorus depending, of course, on adequate renal handling. The mechanism by which phosphorus is excreted is not clear. According to Krane and Potts (1977) there is no convincing evidence for renal tubular phosphate secretion. Knochel (1977) however, states that there is some evidence that tubular secretion of phosphorus might occur but that the importance of this factor as a means to regulate total body phosphorus in humans has not been determined.

Phosphorus reabsorption by the proximal tubule is dependent upon parallel sodium reabsorption. "Therefore the effects of volume expansion and decreased sodium reabsorption are to increase phosphorus clearance, similarly diuretics such as acetazolamide, which act proximally are phosphaturic parallel to the degree to which they are natriuretic" (Krane and Potts 1977, p. 2009).

There is agreement that the kidney is a major organ system that functions to regulate phosphorus concentrations in the body. Current research findings suggest that the regulation of phosphorus homeostasis is extremely complex and involves many body systems. Recent work in the field also suggests that phosphorus regulation is intimately involved with calcium homeostasis. A complete understanding of phosphorus regulation and metabolism must include a discussion of calcium regulation by parathyroid hormone, calcitonin, and vitamin D₃.

**Parathyroid Hormone.** The parathyroid gland is critical in calcium homeostasis. "The physiologic function of parathyroid hormone in man as well as in other mammalian species is to maintain extracellular fluid calcium concentration" (Krane and Potts 1977, p. 2009). When
serum calcium falls, the parathyroid gland is stimulated to release para-thyroid hormone. The goal of this hormone is to increase the serum cal-cium and it does this by (Tepperman 1973, p. 233): "(1) Increasing the rate of resorption of bone mineral, and (2) causing simultaneous events in the intestine and kidney which also have effect of increasing serum calcium." In the intestine the hormone causes increased absorption and in the kidney it causes both increased resorption of calcium and decreased resorption of filtered phosphate. This latter effect leads us to the parathyroid role in phosphorus metabolism.

For a long time it was believed that the most important function of parathyroid hormone was to cause the rapid and immediate loss of phos-phate ion (Guyton 1976). "The phosphaturia, which has the effect of causing an acute decrease in serum PO$_4$$_4$, favors the introduction of more Ca$^{++}$ into the body fluids by decreasing PO$_4$$_4$ inhibition of bone resorp-tion" (Tepperman 1973, p. 234). According to Tepperman, the primary ef-fect of PTH hormone was thought to be PTH-induced phosphaturia until definite data were obtained regarding its effects on bone. Now the hypophosphatemic effects of PTH as well as the phosphaturic effects are considered as parts of the overall coordinate response of the hormone. PO$_4$$_4$ tends to inhibit bone resorption, which means that PTH works better when PO$_4$$_4$ levels are low (Tepperman 1973). The primary effect of para-thyroid hormone is on bone resorption and the phosphaturic effect is of secondary importance (Guyton 1976).

Parathyroid hormone greatly enhances calcium absorption from the intestine. When greater amounts of calcium are reabsorbed, less calcium phosphate is lost in the feces thus increasing the absorption of
phosphorus (Guyton 1976). Tepperman (1973) states that PTH increases Ca++ absorption in the intestine by stimulating the synthesis of the most active derivative of Vitamin D.

The parathyroid gland responds to a decrease in serum calcium concentration by increasing secretion of parathyroid hormone; conversely if serum calcium increases, the parathyroid gland activity decreases (Guyton 1976); and, as already discussed, these effects on calcium metabolism have important effects on phosphate metabolism as well.

**Calcitonin.** Calcitonin, another calcium regulating hormone, secreted by the "C" cells located in the interstitial tissue between the follicles of the human thyroid gland, functions to reduce blood calcium ion concentration (Guyton 1976). Therefore it has an opposite effect to parathyroid hormone. Calcitonin, however, operates much more rapidly, and for a shorter time than parathyroid hormone. The calcitonin system is a very weak one and the long term regulation of calcium ion level extracellularly is almost completely left up to the parathyroid gland (Guyton 1976).

Krane and Potts (1977) describe calcitonin as a hypocalcemic, hypophosphatemic peptide hormone. They explain that the principal action of calcitonin is to block bone resorption.

The importance of calcitonin in increasing urinary calcium and phosphate clearance is synergistic with its effects on bone resorption. The actions of calcitonin on kidney and bone are in turn modulated by the regulation of calcitonin production by serum calcium; hypercalcemia stimulates and hypocalcemia suppresses calcitonin release (Krane and Potts 1977, p. 2011).

**Vitamin D.** Vitamin D is the next critical substance to be discussed in the complex events surrounding phosphate homeostasis.
Norman, Friedlander, and Henry (1977) point out that the most notable advance in understanding Vitamin D and its mechanism of action has been the elucidation of the complex metabolic pathway which has evolved to produce the biologically active form, 1,25-dihydroxyvitamin D$_3$ (1,25(OH)$_2$D$_3$). They also note that there has been a realization that the mechanism of action of the fat soluble Vitamin D is very similar to that of many classical steroid hormones and that in reality Vitamin D is a steroid, a seco steroid.

Haussler (1974, p. 258) states that "because the renal synthesis of 1,25-dihydroxyvitamin D$_3$ is feedback regulated by the calcium needs of animals this metabolite can be classified as a true sterol hormone that mediates calcium transport in target tissues such as intestine and bone." Vitamin D has potent effects on increasing calcium absorption from the intestinal tract as well as effects on both bone deposition and bone reabsorption (Haussler 1974). Wasserman and colleagues established that the generation of a specific protein (calcium binding protein) which has the capability of binding calcium in a highly specific fashion is one of the primary biological responses of Vitamin D or its active forms in the intestine (Wasserman 1969). The exact identities of new proteins induced by active forms of Vitamin D are not known (Tepperman 1973).

Much remains unknown about Vitamin D's specific mechanism of action, such as how it exerts its effects on bone. Guyton (1976) discusses that Vitamin D promotes bone calcification and one way it does this is to increase concentrations of calcium and phosphate in extracellular fluid. But, even in the absence of calcium and phosphate
increases, Vitamin D enhances mineralization of bone. The mechanism of
action, though unknown, is probably related to the ability of 1,25(OH)\textsubscript{2}D\textsubscript{3} to cause transport of calcium ions through cell membranes perhaps
through the osteoblastic or osteocyte cell membranes (Guyton 1976).

Vitamin D also promotes bone resorption and again the mechanism
of action is now known. The ability of Vitamin D to move calcium ions
through cell membranes is probably related to its ability to increase
bone resorption as well as bone calcification (Guyton 1976). The ex-
planation for Vitamin D action appears to be related to its interde-
pendent relationship with parathyroid hormone. It is known that the
conversion of 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol (1,25D\textsubscript{3}) requires parathyroid hormone since in the absence of PTH
either none or almost none of the 1,25D\textsubscript{3} is formed. Norman et al. (1977)
acknowledge that parathyroid hormone has been implicated by a number of
studies as a stimulatory hormone for the production of 1,25(OH)\textsubscript{2}D\textsubscript{3} by
the kidney. After reviewing these studies which have contrasting re-
sults, these authors conclude however, that the role of PTH in the regu-
lation of Vitamin D metabolism is not clearly defined.

Harris and Heany (1969) cited that Vitamin D was required for
the osseous effects of PTH, while Krane and Potts (1977) stated that the
principal action of Vitamin D is to increase the efficiency of intestinal
calcium absorption therefore preventing calcium deficiency. They stated
that although there is no direct proof, there is much speculation that
the vitamin may directly affect bone formation, calcium and/or phosphate
clearance by direct action of metabolites.
"The rate of secretion of parathyroid hormone is controlled almost entirely and very potently by plasma calcium ion concentration" (Guyton 1976, p. 1054). If calcium levels rise, this inhibits parathyroid secretion which in turn inhibits 1,25D₃ formation causing decreased intestinal absorption of calcium and helping return the calcium level to normal (negative feedback) (Guyton 1976).

Krane and Potts (1977) reported that calcium and phosphate ions as well as parathyroid and calcitonin hormones have been reported to affect the efficiency of conversion of 25-HCC to 1,25DHCC.

There is general agreement that low calcium concentration stimulates and high calcium suppresses 1,25-DHCC formation in vivo. However, an important role for phosphate is being stressed (low phosphate, stimulation; high phosphate, suppression of 1,25-DHCC formation). The calcium effects are dependent on functioning parathyroid glands; the phosphorus effects seem independent (Krane and Potts 1977, p. 2012).

**Vitamin D, PTH, Phosphorus.** Physiologically, however, in regulation of the renal metabolism of Vitamin D in normal subjects, PTH seems critical, this may be through effects on phosphate levels in the kidney. Certainly further work is needed to clarify the many important aspects of Vitamin D metabolism (Krane and Potts 1977).

A study done on rats by Garabedian et al. (1976) suggested that 1,25(OH)₂D₃ might be an important factor in serum phosphorus homeostasis. These authors note that it has recently been shown that 1,25(OH)₂D₃ increases serum phosphorus concentration of rats on a low phosphorus diet. They found in their study:

... a bi-directional effect of 1,25-dihydroxycholecalciferol on serum phosphorus in rats. 1,25(OH)₂D₃ injection is followed by an increase in low serum phosphorus concentrations and a decrease in high serum phosphorus concentrations. On the whole and whatever the initial concentration, 1,25(OH)₂D₃
tends to bring serum phosphorus concentrations toward physiological levels, i.e., around 8.5 to 9.5 mg/100ml (Garabedian et al. 1976, p. 797).

This study also showed that the $1,25(OH)_2D_3$ effect on serum phosphorus did not require circulating parathyroid hormone and/or calcitonin (Garabedian et al. 1976).

Other Control. In addition to the three regulatory hormones discussed: parathyroid hormone, calcitonin and Vitamin D, there are other hormones as well that have been shown to effect phosphorus metabolism, such as the pituitary, thyroid, adrenal cortical and gonadal hormones.

Hyperpituitarism, specifically excess growth hormone, results in hyperphosphatemia, whereas hypopituitarism has not been demonstrated to alter phosphorus or calcium metabolism. As early as 1938, Pfeiffer and Gardner demonstrated increased phosphate retention due to the specific action of androgenic substances on protein synthesis (Simpson and Dale 1972, p. 326).

Thus the search goes on not only to clarify the complex interrelationships present in the regulation of calcium and phosphorus homeostasis, but to also clarify the metabolic role of $PO_4$ and alterations and control of $PO_4$ that occur during the menstrual cycle.

Phosphorus and the Menstrual Cycle

Ben-Aryeh et al. (1976) found that salivary phosphate peaked during the periovulatory period. They did not measure serum phosphorus. Simpson and Dale (1972) measured serum phosphorus, calcium, and magnesium during the menstrual cycle and found no significant variation in non-normally contracepting women, nor in the artificially cycled orally contracepting patients.
Reijo Punnonen (1978) measured phospholipids, serum cholesterol, and triglycerides during the menstrual cycle. Punnonen found low levels of phospholipids at various phases of the menstrual cycle. This was in contrast to reports, cited by Punnonen, that phospholipid levels are lowest at ovulation, while still others say phosphorus is highest at ovulation and still others which say there is very little variation (Punnonen 1978).

Erythrocyte 2,3-diphosphoglycerate (2,3DPG) levels were measured during the menstrual cycle with no significant change in its concentration being found. The concentration of 2,3DPG was found to increase in early pregnancy and decrease gradually thereafter (MacDonald and MacDonald 1977).

Pitkin et al. (1978) measured serum calcium, phosphorus, magnesium, parathyroid hormone and calcitonin during the menstrual cycle. They found that the phosphorus levels varied throughout the menstrual cycle but that no particular or significant pattern was present. They also found that parathyroid hormone levels rose progressively through the follicular phase to peak at or slightly before the LH surge, then fell progressively through the luteal phase, while total calcium, magnesium, and phosphorus showed no particular pattern. Ionic calcium tended to fall three to four days before ovulation and then increased. These authors suggest that there is an estrogen effect operating to inhibit parathyroid hormone induced bone resorption. Specifically, as the estrogen rises preovulatory bone resorption is inhibited lowering serum calcium and therefore stimulating parathyroid hormone output. After the decline of the preovulatory estrogen, bone resorption is no longer
inhibited, Ca++ levels rise and parathyroid hormone secretion falls. Other references also cite that estrogen is an inhibitor of bone resorption (Harris and Heany 1969; Heany 1976; Tepperman 1973).

Upon the cessation of menstrual periods at menopause, phosphate levels are said to rise in women (Aitken et al. 1973; Krane and Potts 1977). Takayoshi (1975) studied the effect of menopause on serum levels of calcium and inorganic phosphorus and concluded that the decline in estrogen secretion results in hypercalcemia and hyperphosphatemia. Takayoshi suggests that prophylactic estrogen therapy be considered at the early stage of the postmenopause for preventing the increased bone resorption. Aitken et al. (1973, p. 593) found that "mean serum phosphorus concentration was significantly higher after menopause and was significantly lower in those women on long-term dose menstranol therapy. A significant correlation was found between serum phosphorus and plasma human growth hormone concentrations in untreated postmenopausal women."

These authors suggest that many changes in bone mineral homeostasis "and the relative hyperphosphatemia in particular are consistent with a postmenopausal increase in human growth hormone activity" (Aitken et al. 1973, p. 597).

In summary, phosphorus is an integral and critical constituent of all body tissues, essential for the structure and function of all cells, all synthetic as well as many catabolic processes. Phosphorus plays a role in the complex events surrounding the control of the menstrual cycle. The exact nature of that role remains to be determined.
CHAPTER 3

METHODOLOGY

The material presented in this chapter is the research design, description of the sample, data collection, laboratory analysis, and analysis of data.

Research Design

A descriptive design was utilized in order to identify the pattern of serum phosphorus levels during the menstrual cycle and specifically to identify whether there was a significant periovulatory period increase in serum phosphorus in ovulating women.

Description of the Sample

The serum utilized in this study for phosphorus analysis was collected for another study for analysis of serum sodium and potassium and steroid hormones in ovulating women during the menstrual cycle (Voda 1976). The following description of the sample population is taken from Voda's dissertation (1976, p. 14).

Volunteers were interviewed to assess their general health, to assess the regularity and normalcy of menstrual cycles, and to determine the use of chemical and mechanical contraceptive devices. Twenty-four menstruating women ranging in age from 19 to 36 years, not using oral contraceptives, were selected as subjects. Data were collected every other day and subjects were arbitrarily divided into two groups. This division allowed the investigator to handle the volume of work; that is twelve subjects reported to the investigator on alternate days over a 32-day period. Based upon information provided by subjects on
interview, a 32-day data collection period was considered adequate for obtaining data on all subjects in all phases of one menstrual cycle. No attempt was made to minimize stress or to control diet or activity of the subjects (Voda 1976, p. 14).

A convenient serum sample from 11 of 24 subjects was available for phosphorus analysis of this study.

Data Collection

The following information on data collection was taken with permission from Voda's dissertation (see Appendix A):

Data were collected early in the morning and most subjects came directly to the laboratory from their homes. All subjects had appointments for the same time in the morning of their data collection day. Before collection was started, the subjects read and signed a subject consent form. This form stated the subject's responsibility to the project and the investigator's responsibility (Voda 1976, p. 15).

Collection of Blood

Blood was collected from each subject every other day. A minimum of 10 ml. of blood was collected from each subject by venipuncture of arm veins using vacutainer technique. Each blood sample was immediately stored at 4 degrees C. and serum was separated soon after collection. The clot was rimmed, centrifuged, and serum aliquots were pipetted into plastic and glass vials. About 0.3 ml. of serum were transferred with disposable Pasteur pipettes into plastic vials for electrolyte analysis; the remainder of the serum was pipetted into glass vials to be used for osmolality and steroid radioimmunoassay. Aliquots were stored frozen at -20 degrees C. (Voda 1976, p. 17).

(The frozen serum samples from 11 of 24 subjects were available for phosphorus analysis.)

Permission was obtained from Human Subjects Committee in 1974 by Voda to collect human serum for electrolyte analysis (see Appendix B).
Indirect Indicators of Ovulation

1. Basal Body Temperature

Subjects were given oral thermometers and instructed in measuring oral basal body temperature. Subjects took their own temperatures upon awakening, prior to getting out of bed, recorded it and brought the record to the investigator. All instances of infection, lack of sleep and so forth were recorded on the day or days they occurred (Voda 1976, p. 15).

2. Serum Progesterone

Serum progesterone was measured by radioimmunoassay (Voda 1976, p. 19).

Laboratory Analysis

Serum Phosphorus Measurement

Pierce 47400 Phosphorus Rapid Stat kit (Pierce Chemical Company, Rockford, Illinois) was utilized in measuring serum phosphorus. The Phosphorus Rapid Stat procedure was followed as outlined in the handout accompanying the kit (Pierce Chemical Company 1976). The procedure follows a method described by Henry (1964) which involves the reduction of phosphomolybdate to molybdenum. Fresh standards of 0, 4.0, 10.0, and 20.0 were prepared daily according to instructions provided in the Phosphorus Rapid Stat kit. The Spectronic 710 spectrophotometer (Bausch and Lomb Company) was calibrated in the 0 to 20.0 mg/dl range and a calibration graph was prepared in order to check the linearity of the response of the instrument. The accuracy of the assay procedure was assured by daily calibration of the spectrophotometer, preparation of fresh standards daily, using pooled serum for internal control and the preparation of samples in duplicate for assay.
Statistical Analysis of Data

The Tektronix 4051 Graphics Display Computer was utilized to compute mean phosphorus values for eleven subjects per data collection day over a 32-day period. These computed means were plotted on a x, y axis, menstrual cycle day versus serum phosphorus values. Measures of dispersion were determined and plotted in the same manner as the mean using the Tektronix 4051 Computer. The mean and standard deviation were calculated to determine the central tendency and range of variability of the serum phosphorus levels for the eleven subjects over the 32-day period.

Data were analyzed on a Tektronix 31 Calculator using a program described in 31 Statistics Program Library Manual (Tektronix 1973) in order to compute the student t-test. The student independent t-test was utilized to determine whether there was a difference between serum phosphorus levels in the periovulatory period versus the menstrual phase, the periovulatory period versus the follicular phase and the periovulatory period versus the luteal phase.
CHAPTER 4

RESULTS

This chapter presents the analysis of the data and the characteristics of the sample.

Subject Selection Based on Biphasic Basal Body Temperature Curve and Serum Progesterone Increase

Nine of eleven subjects whose serum phosphorus levels were analyzed met indirect criteria for ovulation. They demonstrated biphasic basal body temperature (BBT) curves (Haller 1972) associated with serum progesterone levels that were 3.0 ng/ml or more in the midluteal phase of their cycles. According to Israel et al. (1972) this midluteal phase increase of serum progesterone is presumptive evidence that ovulation has occurred.

The serum progesterone and biphasic basal body temperature curves for nine ovulating subjects are shown in Figure 1.

Two of eleven subjects did not meet both of the above ovulation criteria and were classified as non-ovulators. The serum progesterone and basal body temperature curves for these subjects are shown in Figure 2.

The raw data on serum phosphorus for eleven ovulatory subjects is found in Appendix C.
Figure 1. Progesterone and Basal Body Temperature for Nine Ovulating Subjects Over a 32-Day Period
Figure 2. Progesterone and Basal Body Temperature for Two Non-ovulating Subjects Over a 32-Day Period
Serum Phosphorus in Ovulating Women

No statistically significant periovulatory period peak in serum phosphorus levels for ovulating subjects was found. Results of the t-tests comparing serum phosphorus values during the menstrual, follicular and luteal phases of the menstrual cycle with serum phosphorus values during the periovulatory period phase are shown in Table 1.

Table 1. Student Independent t-test Comparing Serum Phosphorus Values During Menstrual, Follicular and Luteal Phases with Serum Phosphorus Values During the Periovulatory Phase for Nine Ovulating Subjects

<table>
<thead>
<tr>
<th>Comparisons</th>
<th>Degrees of Freedom</th>
<th>t Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menstrual Phase versus Periovulatory Phase</td>
<td>53</td>
<td>-0.4089</td>
<td>NS</td>
</tr>
<tr>
<td>Follicular Phase versus Periovulatory Phase</td>
<td>53</td>
<td>0.2901</td>
<td>NS</td>
</tr>
<tr>
<td>Luteal Phase versus Periovulatory Phase</td>
<td>81</td>
<td>9.0586</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = Not statistically significant (p > 0.05)
Phosphorus data for each ovulatory subject throughout the menstrual cycle are shown in Figures 3-11. Subjects A, M and O had the smallest range of phosphorus values throughout the menstrual cycle (see Figures 3, 8, and 10). Subject D had the greatest range in phosphorus values throughout the menstrual cycle with a low of 3.21 mg/dl and a high of 5.97 mg/dl (see Figure 4). There were fluctuations throughout the menstrual cycle for subjects E, F, H, N, and U but with no particular pattern or extreme range (see Figures 5, 6, 7, 9, and 11). The range of phosphorus values during the periovulatory period for ovulating subjects was 2.60 mg/dl to 5.65 mg/dl.

A composite picture of all ovulating subjects (n=9) is shown in Figure 12. As noted earlier, no statistically significant periovulatory period in serum phosphorus levels for n=9 was found. The mean value for serum phosphorus levels (n=9) on the estimated day of ovulation was 3.88 mg/dl while the standard deviation was ±0.75 mg/dl. Since all subjects did not ovulate on the same day of their menstrual cycle the data were manipulated so that the estimated day of ovulation is the same point in time for all subjects. Thus variability in values and overlap of phases increase as one moves to the left or right of the estimated day of ovulation.

Serum Phosphorus in Non-ovulating Women

Subjects R and I showed slight increases in serum progesterone at about mid-cycle, but progesterone did not exceed 3 ng/ml, and they were considered as non-ovulators.
Figure 3. Pattern of Serum Phosphorus for Subject A Over a 32-Day Period
Figure 4. Pattern of Serum Phosphorus for Subject D Over a 32-Day Period
Figure 5. Pattern of Serum Phosphorus for Subject E Over a 32-Day Period
Figure 6. Pattern of Serum Phosphorus for Subject F Over a 32-Day Period
Figure 7. Pattern of Serum Phosphorus for Subject H Over a 32-Day Period
Figure 8. Pattern of Serum Phosphorus for Subject M Over a 32-Day Period
Figure 9. Pattern of Serum Phosphorus for Subject N Over a 32-Day Period
Figure 10. Pattern of Serum Phosphorus for Subject 0 Over a 32-Day Period
Figure 11. Pattern of Serum Phosphorus for Subject U Over a 32-Day Period
Figure 12. The Mean and Standard Deviation of Phosphorus for Nine Ovulating Subjects Over a 32-Day Period
No phosphorus peak was observed in subjects R and I. There were fluctuations in phosphorus levels throughout the cycle ranging from 0 to 1.37 mg%. The phosphorus data for subjects R and I is shown in Figures 13 and 14.
Figure 13. Pattern of Serum Phosphorus for Subject R Over a 32-Day Period
Figure 14. Pattern of Serum Phosphorus for Subject I Over a 32-Day Period
CHAPTER 5

DISCUSSION AND RECOMMENDATIONS

Discussion

The objective of this thesis was to measure serum phosphorus levels across one menstrual cycle so as to determine whether phosphorus levels changed during the periovulatory period in ovulating women. The study was significant to nursing in that it added to the body of scientific knowledge of the menstrual cycle, contributed to the search for a safe, effective and indirect indicator of ovulation, and provided information regarding effects of hormonal interaction on nutritional needs.

The findings of this study showed that there were fluctuations in phosphorus levels throughout the menstrual cycle, but no significant serum phosphorus periovulatory peak was found. These findings are consistent with those reported by Simpson and Dale (1972), and Pitkin et al. (1978), who found no significant variation in serum phosphorus levels.

The findings of the present study differ from the findings of Ben-Aryeh et al. (1976) who analyzed phosphorus in saliva. The results of this study cannot be compared with the salivary findings of Ben-Aryeh's in that two different body components were studied. However, the fact that phosphorus in sputum was found to peak during the periovulatory period while no significant fluctuations were found in serum phosphorus raises questions regarding phosphorus metabolism during the menstrual
cycle and particularly the role of salivary glands in the regulation of serum phosphorus.

It is of note that although no significant periovulatory period change was found in serum phosphorus in the present study, six of nine ovulating women showed a slight nonsignificant decrease in serum phosphorus at estimated time of ovulation, ranging from 0.08 to 1.45 mg/dl with a mean of 0.47 mg/dl, followed by luteal phase increase in serum phosphorus associated with progesterone rise. Conversely, the two anovulatory women showed a slight nonsignificant increase in serum phosphorus at midcycle which preceded the progesterone rise. The absence of the serum phosphorus peak in this study versus the salivary peak found by Ben-Aryeh et al. (1976) suggests that there may be increased phosphorus activity in metabolic processes around ovulation and that salivary glands, perhaps via hormonal control of active secretion into saliva, may function to maintain serum phosphorus within normal limits. This hypothesis needs to be explored.

Phosphorus is involved in the regulation of the menstrual cycle as a component of cyclic-3'5'-adenosine monophosphate (Cyclic AMP). In the formation of cyclic AMP, a phosphorus compound, adenosine triphosphate (ATP), is catalytically converted to cyclic AMP with the release of two phosphate molecules. Phosphorus compounds are metabolized and released particularly around ovulation. Specifically, cyclic AMP is involved initially in the release of gonadotrophic releasing hormones from the hypothalamus; second cyclic AMP mediates the release of gonadotropins from the anterior pituitary. LH acting as "first messenger" travels to the ovary, binds to specific receptor sites on the outside of the
ovarian cell membrane, which then is followed by the catalytic conversion of ATP to cyclic AMP. Large amounts of ATP are catabolized in this process. Cyclic AMP acts as second messenger. It diffuses throughout the cell instructing it to respond in a characteristic way. For example, due to the action of the hormone LH as the first messenger and the hypothesized action of the second messenger cyclic AMP, the ovary is stimulated to synthesize and secrete gonadal steroids. ATP and its catalytic conversion to cyclic AMP, play an integral role in the regulatory activities that both precede and follow ovulation.

Phosphorus is a critical constituent of all body tissue and normal phosphorus levels are essential to normal body function. Dietary intake of phosphorus, absorption of phosphorus from the intestine as well as very efficient recycling of phosphorus by the kidney have been discussed as important factors in maintaining normal phosphorus levels. The body recognizing the need for an adequate yet not excessive level of phosphorus attempts to maintain that level through a complex set of mechanisms involving many body systems, including renal, gastrointestinal, and endocrine. All of the phosphorus regulatory mechanisms are not completely understood and at the time of this writing some remain hypothetical. A function for the salivary glands in the regulation of phosphorus during the menstrual cycle is possible because of the periovulatory period findings of a phosphorus peak by Ben-Aryeh et al. (1976) while serum levels appear to remain stable. It is hypothesized that salivary glands have estrogen receptors and during estrogen rise in serum the enzymes in the salivary gland (such as alkaline phosphatase) are activated and change the secretion of phosphate. Further work to clarify
this regulation and metabolism during the menstrual cycle is recom-
mended.

Pitkin et al. (1978) found that parathyroid hormone levels rose progressively through the follicular phase to peak at or slightly before the LH surge, then fell progressively through the luteal phase. Ionic calcium tended to fall three to four days before ovulation and then to increase. These authors explain these changes on the basis of estrogen; they hypothesize that estrogen inhibits PTH induced bone resorption. So, as estrogen rises preovulatory, bone resorption is inhibited lowering serum calcium which stimulates PTH secretion. PTH is known to cause phosphaturia (Guyton 1976; Tepperman 1973), therefore if PTH levels are peaking periovulatory there should be a concomitant urinary phosphorus increase unless some other mechanism is operating to counteract large losses, such as salivary secretion. Once the phosphorus is secreted by the salivary glands it could be reabsorbed through the GI tract and thus recycled.

Recommendations

This author recommends that a study be done with a larger sample of ovulatory and anovulatory women throughout one menstrual cycle to describe the pattern of phosphorus in serum, sputum, and urine. This type of study could be extremely informative in terms of furthering our understanding of phosphorus regulation, its relationship to the menstrual cycle, as well as provide insights and a base to better understand the changes in mineral metabolism that some women experience at menopause. Since both urine and sputum are noninvasive sources, substances found
in these sources might potentially be utilized as indirect indicators of ovulation. Phosphorus is easily analyzed in both urine and sputum, is involved in the regulatory aspects of the menstrual cycle and is a promising substance to investigate in the search for an indirect indicator of ovulation.
APPENDIX A

LETTER PERMITTING EXTENDED QUOTATION

FROM VODA DISSERTATION
Dear Mrs. Wilde,

You have permission to quote extensively from my dissertation "Correlations Among Physiological Indicators, Physical Signs and Subjective Complaints in Premenstrual Edema," dated 1976.

Sincerely,

Anna M. Voda
Associate Professor

AMV:ka
APPENDIX B

HUMAN SUBJECTS COMMITTEE APPROVAL LETTER
Ms. Anna M. Voda
Animal Physiology Program
Dairy and Food Science
College of Agriculture
Bldg. 38
Campus

Dear Ms. Voda:

The Human Subjects Committee has approved your proposal entitled
"Investigation of Relationships among Fluctuating Menstrual Cycle
Hormones, Electrolytes, Osmolality, Body Weight, Finger and Ankle
Measurements and Symptoms of Premenstrual Swelling in Women during the

The Human Subjects Committee is available to consider any problems
which might arise with regard to the use of human subjects, and further
you are advised that any changes from the procedures proposed in your
proposal as approved require review by the Committee. You must also
report to the Committee any physical or psychological injury to the
subjects which results from their participation in the project.

If we can be of further assistance in this or other matters please
feel free to call or write.

Sincerely,

Thomas Weaver, Ph.D.
Chairman
Human Subjects Committee

cc: Dr. Kassander
### APPENDIX C

**RAW DATA FOR ELEVEN SUBJECTS**

**Serum Phosphorus Values mg/dl**

<table>
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<th>Data Collection Day</th>
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<th>Subject D</th>
<th>Subject E</th>
<th>Subject F</th>
<th>Subject H</th>
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