Effect of Fasting and Parathyroid Hormone Injection on Plasma $^{45}$Ca Concentrations in Rats

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Young male rats were administered $^{45}$Ca 5 days to 2 weeks prior to use. All rats were either parathyroidectomized (PTX) or thyroparathyroidectomized (TPTX) and given several days to recover from surgery. The first group of rats were maintained on a 12 h dark-fed and 12 h light-fasted daily cycle. The remainder of the rats were used for parathyroid hormone (PTH) studies (0.1–0.6 U/g body weight) following which blood samples were obtained from the tail for 1 to 6 h. Two groups of these rats were bilaterally nephrectomized 18 h before PTH injection. Two contrasting results were obtained: in PTX (or TPTX) rats maintained on the closely regulated food and light regime, plasma $^{45}$Ca concentrations rose markedly each day at the start of the fasting period and then fell slowly. Total plasma calcium values fell throughout the fasting period. A similar rise and fall was also observed in $^{45}$Ca values of rats experimentally fasted after being maintained with food continuously available. In contrast, in all PTX or TPTX rats, PTH injection was followed by an equal rise in both plasma calcium and $^{45}$Ca values so that for the first few hours plasma $^{45}$Ca specific activity was unchanged. These data are consistent with the concept of a bone fluid compartment (BFC) separated by a cellular interface from the primary extracellular fluid space (ECF). It is postulated that through this cellular interface calcium is actively 'pumped' from the BFC to the ECF. The rise in plasma $^{45}$Ca values at the start of fasting is explained on the basis of decreased entry of stable calcium from the gastrointestinal tract and a continued movement of calcium and $^{45}$Ca from the BFC to the ECF. The concomitant increase in plasma calcium and $^{45}$Ca during the first few hours after PTH injection is explained by a rapid action of PTH to increase the rate of calcium movement from BFC to ECF by its action at the cellular interface, without altering $^{45}$Ca specific activity until such time as dissolution of bone crystals is required as a supply of calcium.

Key words: Bone — Calcium — Homeostasis — Parathyroid hormone — $^{45}$Ca.

One of the well-confirmed actions of parathyroid hormone (PTH) is to increase the rate of removal of calcium from bone (McLean and Urist, 1955). Both in vivo and tissue culture studies have provided evidence that PTH administration is followed by increased bone breakdown, apparently due predominately to osteoclastic action (Heller et al., 1950; Raisz, 1965). In our previous reviews (Talmage, 1969; Talmage et al., 1970) we have suggested two separate actions of PTH in bone: 1) to maintain plasma calcium concentrations by rapidly increasing calcium transport from bone and 2) to slowly increase bone resorption. While bone breakdown appears to provide additional calcium for the extracellular fluid over long periods, the calcium transport system responds rapidly to provide for the minute-to-minute regulation of the plasma calcium level.

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Although it is recognized that calcium may be transferred directly from the solid phase of bone into the primary extracellular fluid space (ECF) by osteoclastic action, our working hypothesis has been that there is a bone fluid compartment (BFC), and that the primary calcium fluxes between the ECF and bone involve these two fluid compartments (Talmage, 1969). The concept of BFC is not new (Howard, 1956), and has received experimental support by the work of Neuman and his associates (Strates et al., 1957; Triffitt et al., 1968; Neuman and Ramp, 1971).

Previously we provided experimental evidence for the existence of a mechanism for rapid changes in calcium transport from bone in studies on mice in which bone was prelabeled with $^{45}$Ca and $^{32}$P (Meyer and Talmage, 1972). Our findings that in the first few hours after PTH administration, the hormone influenced $^{45}$Ca removal from bone without concurrent effects on $^{32}$P removal suggested that the plasma calcium raising effect of PTH preceded an effect on bone mineral breakdown.

The present studies were designed to examine the early action of PTH in parathyroidectomized (PTX) and thyroparathyroidectomized (TPTX) rats which had been prelabeled with $^{45}$Ca and/or $^{32}$P. In a recent study (Talmage et al., 1975) we observed that plasma levels of the radionuclides in intact rats fluctuates during each 24 h period. These studies demonstrated that in rats in which the radioisotopes had been administered 5 days to 1 month prior to the experimental study, there were regular cyclic changes in plasma concentrations of the nuclides, affected by feeding schedules, in which the maximum values (late afternoon) were almost double those of the minimum values (early morning). In the experiments to be described in this report, the daily change in plasma radionuclides are reported for PTX rats. Additionally, the early effects of PTH were examined. Due to the scope of the problem, only the effects of PTH on $^{45}$Ca changes are reported here.

Materials and Methods

Over 100 male Zivic-Miller rats (weight range 150-250 g) were used in these experiments. For the diet studies, the rats were placed on a controlled light and food schedule: ‘dark’ period from 7 p.m. to 7 a.m.; food was provided only during this period. Food consisted of standard Purina lab Chow for all experiments.

Blood samples (approximately 0.25 ml each) were obtained from the tail vein and analyzed for calcium (Hill, 1965), phosphate (Chen et al., 1965) and $^{45}$Ca. In the dietary experiments, rats were bled a maximum of 3 times in any one day and only once during a feeding period. Radiocalcium was administered intraperitoneally at times indicated in the individual experiments ($^{45}$Ca S.A. = 16 mCi/mg).

Surgery was performed under light ether anesthesia. Either the parathyroids were removed individually (PTX), or the entire thyroparathyroid complex was removed (TPTX). For extended maintenance after removal of the thyroid, thyroxine (5 µg/100 g body weight) was administered twice weekly. When the rats were nephrectomized (Nephx) both kidneys were removed through a single midventral incision.

Parathyroid hormone (TCA-powder 160 USP U/mg) was obtained from Wilson Laboratories. The dose used varied from 0.2-0.6 U/g body weight and was injected subcutaneously.

Results

Daily Changes in Plasma Calcium and $^{45}$Ca in (T)PTX Rats

Two groups of rats prelabeled with $^{45}$Ca for 5 or more days were used to determine daily plasma calcium and $^{45}$Ca fluctuations in the absence of the