was used for the fibronectin receptor (*14*). Our approach with electrostatically complementary peptides is different from either of these techniques.

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8. The amount of specific antibody present in the preparations was determined by quantitative immunoprecipitation with peptide-conjugated ^{125}I-labeled ovalbumin (the average peptide-to-ovalbumin molar ratio was 5:1 as determined by amino acid analysis). The amounts of specific antibodies to the peptides DDDED and EEEDE (anti-DDDED and anti-EEEDE) in the preparations were 22.4% and 64.8%, respectively. The counts (cpm) of ^{125}I-labeled EEEDE-ovalbumin immunoprecipitated by anti-DDDED were about 65% of those by anti-EEEDE. On the other hand, the counts of ^{125}I-labeled DDDED-ovalbumin immunoprecipitated by anti-EEEDE were about 3.5% of those by anti-DDDED. These values were obtained by subtracting the value of control normal rabbit IgG.
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10. Cellular incorporation of [^{3}H]uridine, as assayed by autoradiography, indicated that the injection of anti-DDDED into the cytoplasm did not block the synthesis or release of RNA. Passive diffusion of the monomeric form of phycoerythrin (30 kD) was also unaffected by antibody.
11. Nucleoplasmin or SV40 T–BSA was injected at various times after injection of anti-DDDED (7 mg/ml) into the cytoplasm of cells into which anti-DDDED was injected, and 30 min later, the cells were treated as described in Fig. 3. Complete inhibition of nuclear transport was observed for 3 hours and then the inhibitory activity of the antibody in the cells gradually decreased. These results were supported by the fact that when anti-DDDED was injected into the cytoplasm it remained in the cells for about 3 hours and then gradually disappeared, as shown by injecting the antibody and then examining the cells by indirect immunofluorescence microscopy with goat anti-rabbit IgG.
12. From this and other gels, the sizes were calculated to be 69 and 59 kD. Although the DDED sequence is found in lamins A and C (amino acid positions 552 to 555) [F. D. McKeon, M. W. Kirshner, D. Caput, *Nature* **319**, 463 (1986)], these two proteins had molecular sizes slightly different from lamins A and C and anti-DDDED did not react with lamins A and C by immunoblotting assay under our conditions. Thus, these two proteins are distinct from lamins A and C.
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15. We used three karyophilic proteins such as nucleoplasmin and BSA conjugated with a synthetic peptide containing the nuclear localization signal sequence for SV40 large T antigen (SV40 T–BSA) or polyoma virus large T antigen (polyoma T–BSA). Polyoma T–BSA as well as nucleoplasmin and SV40 T–BSA entered the nucleus effectively when injected into the cell cytoplasm.
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Expression of a Calcium-Mobilizing Parathyroid Hormone–Like Peptide in Lactating Mammary Tissue

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A survey of rat tissues by RNA analysis, aimed at uncovering the physiological function of the parathyroid hormone–like peptide (PTH-LP) associated with hypercalcemia of malignancy, revealed the presence of a 1.5-kilobase messenger RNA encoding this peptide in lactating mammary glands. PTH-LP messenger RNA is expressed in mammary tissue only during lactation; it appears and disappears rapidly (2 to 4 hours) as a function of the sucking stimulus. The identity of this messenger RNA was confirmed by cloning the rat PTH-LP complementary DNA, which predicts a peptide with strong similarity to the human homolog. Moreover, extracts from lactating mammary tissue stimulated parathyroid hormone–dependent adenylate cyclase. These findings suggest that PTH-LP plays a physiological role in lactation, possibly as a hormone for the mobilization or transfer (or both) of calcium to the milk.

A PARATHYROID HORMONE–LIKE PEPtide produced by many tumors from patients with humoral hypercalcemia of malignancy (HHM) (*1*) was recently characterized. Amino-terminal sequence analysis (*2–4*) and subsequent cDNA cloning (*5–7*) revealed a peptide of approximately 16 kD, with amino-terminal homology to PTH. A synthetic peptide containing amino acids 1 to 34 of the human PTH-LP produces the same effects on renal and osteoblast membranes in vitro and on calcium and inorganic phosphate fluxes in vivo (*8, 9*) as the peptide 1–34 of human PTH, thus explaining the resemblance of the HHM syndrome to hyperparathyroidism. In addition to tumors, the only other cells reported to produce PTH-LP are human keratinocytes in culture (*10*); however, the physiological function of the peptide is unknown.

To investigate further the expression and function of PTH-LP, we screened RNAs from adult and embryonic rat tissues for hybridization to a human PTH-LP cDNA (Fig. 1). Among all the normal tissues examined, relatively high levels of a 1.5-kb PTH-LP mRNA were detected only in RNA from lactating mammary tissue. However, similar levels of this mRNA were seen in RNA from rat Leydig cell tumor, which secretes a PTH-LP and causes hypercalcemia in rats (*11*). Skin and spleen gave weak hybridization signals; however, the actual production and possible function of the peptide in these tissues remain to be elucidated. No PTH-LP mRNA was detected in RNA from nonlactating mammary gland, in RNAs from brain, testis, intestine, kidney, liver, lung, parathyroid-thyroid gland, rat osteosarcoma (ROS 17/2.8), and heart of nonlactating animals, or in RNAs from these tissues in lactating animals. Moreover, this mRNA was not detected in rat embryos, in placenta, or in kidney and skin of newborn rats. Thus, we have no evidence to support a role for this peptide in development, although we cannot exclude the possibility that small amounts of this mRNA may be expressed in specific embryonic tissues, since whole embryo RNA was used for this analysis.

Further study showed that the expression of PTH-LP mRNA in mammary tissue was

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closely related to lactation. PTH-LP mRNA was not detected in nonlactating or developing mammary tissue during pregnancy (Fig. 2). However, on the first day postpartum, when suckling had begun, PTH-LP mRNA was clearly present in the dams' mammary tissue. With suckling litters present, mammary tissue PTH-LP mRNA reached the highest concentration at approximately 24 hours postpartum and was maintained at about half that concentration from day 2 to day 20 postpartum. Mammary tissue PTH-LP mRNA became undetectable 24 hours after litters were removed.

To further define the temporal relation between PTH-LP mRNA expression and lactation, we removed suckling litters at 2 days postpartum and dissected the mammary tissue of the dams at 30 min or at 1, 2, 3, or 4 hours later. PTH-LP mRNA decreased sharply between 1 and 2 hours after removal of the pups and became undetectable after 4 hours (Fig. 2). In another experiment, pups were removed from their mothers for 4 hours and were returned for 4 hours, during which time they were observed to be suckling. Mammary tissue from these dams contained PTH-LP mRNA levels comparable to those seen in tissues of other lactating dams at the time these pups were removed. Finally, dams that had their litters removed 2 days earlier were given surrogate litters to allow us to determine whether mammary tissue PTH-LP mRNA expression was related to milk production or to sucking per se. For 3 days the litters were suckling. However, upon dissection, the mammary tissue of the dams had atrophied, and the pups' digestive tracts were filled with air, indicating that in this case sucking did not stimulate milk production. Mammary tissue from these dams did not contain detectable levels of PTH-LP mRNA. Thus, lactating mammary tissue appears to be required for PTH-LP expression.

We confirmed that this mRNA encodes a PTH-LP by using human PTH-LP cDNA to isolate a corresponding rat cDNA (prPLP1). The rat cDNA encodes a peptide of 177 amino acids, consisting of a 36-residue putative prepro-leader sequence followed by the 141–amino acid residue PTH-LP (Fig. 3). The first 111 amino acids of the rat PTH-LP, which include the PTH-like amino-terminal domain, are identical to the human PTH-LP except for two substitutions ($Ala^{49} \rightarrow Ser^{49}$ and $Arg^{98} \rightarrow Lys^{98}$). Since the amino-terminal residue of rat PTH is alanine, PTH-LP shares 9 of the first 13 amino acids with rat PTH, as compared to 8 of 13 with human PTH. This finding could explain why human PTH-LP is more potent than human PTH in rat systems (*8, 9*).

The rat and human PTH-LPs diverge considerably in the carboxyl-terminal domain, where only 8 of the last 30 amino acids are identical. Although fragment 1–34 of PTH-LP produces virtually all known effects of PTH, conservation of residues 35 to 111 between rat and human PTH-LP suggests that this domain may have biological importance. Strong conservation is seen not only in the coding sequence, but also in the 3′ untranslated sequences which are about 90% identical to one (*5*) of the two alternatively spliced (*7*) human PTH-LP mRNAs. The conserved AT-rich motifs present in this region may account for the rapid

Table 1. The stimulation of PTH-sensitive adenylate cyclase by lactating mammary tissues. Tissues from lactating dams or from dams that had their pups removed after birth were obtained 24 hours postpartum. Tissue extracts were prepared essentially as described (*25*). PTH-sensitive adenylate cyclase was assayed by the method of Rodan *et al.* (*13*). The values are means ± SEM of at least three experiments. ND, not determined.

Extract (μg)	^{3}H-labeled cAMP (counts per well in 8 min)		
	Non-lactating	Lactating	With PTH antagonist*
0	231 (±24)		361 (±49)
10	238 (±21)	263 (±16)	ND
25	222 (±5)	348 (±31)	ND
50	226 (±20)	509 (±41)	334 (±15)
100	271 (±11)	670 (±110)	331 (±29)

*The PTH antagonist [D-Trp-12,Tyr-34]bovine PTH-(7–34)NH_2 (*26*) at 4 μ*M* was used in these experiments.

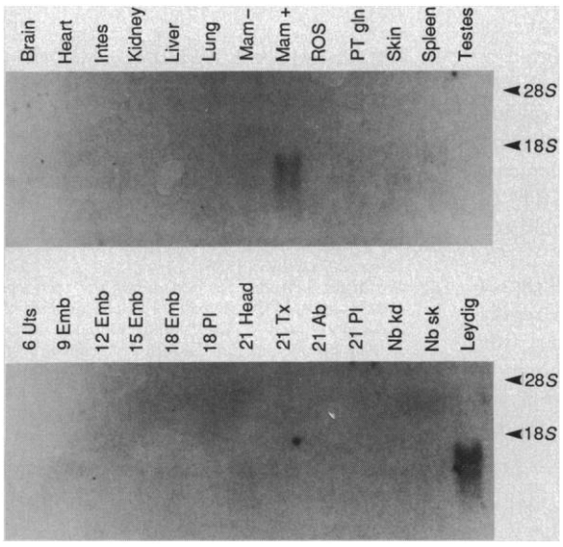

Fig. 1. Tissue distribution of rat PTH-LP mRNA. (**Top**) Each lane contains total RNA (20 μg) from the indicated tissues. Abbreviations: Intes, intestine; Mam−, nonlactating mammary gland; Mam +, lactating mammary gland; ROS, ROS 17/2.8 osteosarcoma cells; and Pt gln, parathyroid gland. (**Bottom**) RNA was extracted on the day of gestation indicated by the numbers above each lane. Abbreviations: Uts, uterus; Emb, embryo; Pl, placenta; Tx, thorax; Ab, abdomen; Nb kd, newborn kidney; Nb sk, newborn skin; and Leydig, Leydig cell tumor. Total RNAs were prepared from rat tissue by the procedure of Chirgwin *et al.* (*22*), separated by electrophoresis on a 1% agarose gel containing 0.66*M* formaldehyde, and transferred to nitrocellulose (*23*). The filters were hybridized at 42°C in 50% formamide, 6× saline sodium citrate (SSC) (1× SSC is 0.15*M* sodium chloride and 0.015*M* sodium citrate, *p*H 7), 5× Denhardt's solution, and sheared herring sperm DNA (0.1 mg/ml). The hybridization probe used was the 408-bp Ava I fragment of the human PTH-LP cDNA 10B5 (*7*). Filters were hybridized for 20 hours in the same solution containing 1×10^6 cpm of the ^{32}P-labeled DNA per milliliter of solution. The filters were washed once for 20 min in 0.1× SSC and 0.5% SDS at 65°C. Autoradiography was done for 10 days at −70°C with two intensifying screens. Uniform loading of RNA in each lane was monitored by ethidium bromide staining of the 28*S* and 18*S* ribosomal RNAs.

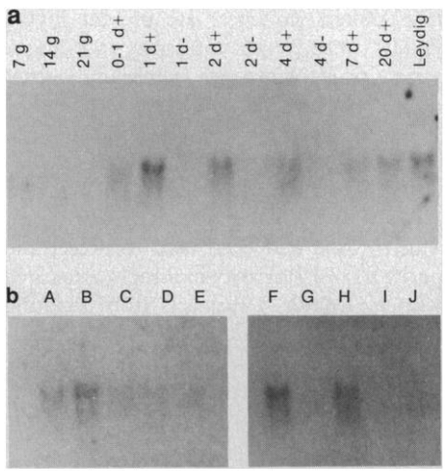

Fig. 2. (**a**) Rat PTH-LP mRNA in lactating and nonlactating mammary tissue. Total RNA (20 μg) from rat mammary tissue was hybridized to the human PTH-LP cDNA under the conditions described in the legend of Fig. 1. The g indicates the day of gestation; d indicates the day postpartum with (+) or without (−) litters. Leydig indicates Leydig cell tumor. (**b**) PTH-LP mRNA is expressed in rat mammary tissue in response to suckling. Mammary tissue RNA was extracted at the following times after the litter was removed: (lane A) 30 min, (lane B) 1 hour, (lane C) 2 hours, (lane D) 3 hours, or (lane E) 4 hours. RNA from mammary tissue at 2 days postpartum: (lane F) with the litter, (lane G) with the litter removed for 4 hours, (lane H) with the litter removed for 4 hours and then returned for 4 hours, (lane I) with litter removed for 8 hours. (Lane J) Mammary tissue RNA from a mother that for 2 days was without a litter and then was given a surrogate litter for 3 days.

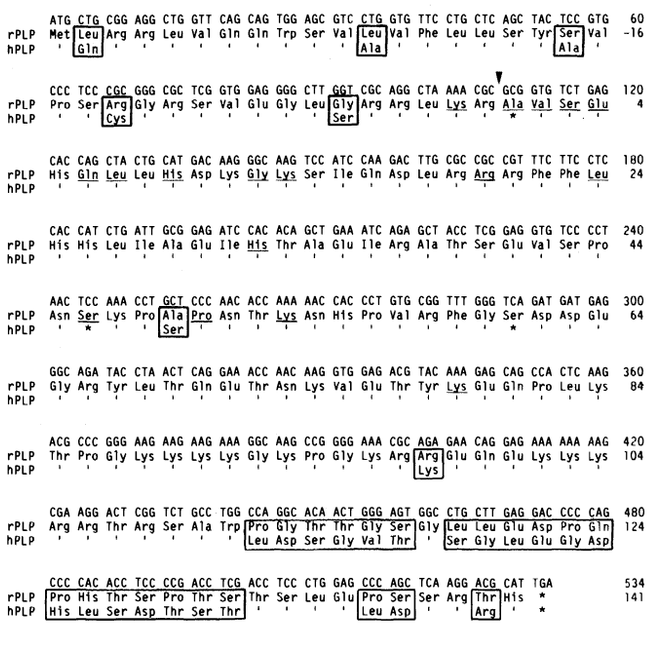

Fig. 3. Nucleotide and predicted amino acid sequence of the rat PTH-LP (rPLP). The 177–amino acid open reading frame begins with a 36–amino acid putative pre-pro-leader sequence. The arrow identifies the putative cleavage point between the leader and PTH-LP. Underlined residues are shared between rat PTH-LP and rat PTH; amino acid differences between rat and the human (hPLP) peptide are boxed. Asterisks designate residues common to either human PTH or rat PTH but not both. A λgt10 cDNA library was prepared from Leydig cell tumor poly-adenylated RNA essentially as described (7). Portions of the library were probed with the human cDNA fragment described in the legend to Fig. 1. The human probe (408 bp) contains only 45 base mismatches between nucleotides 1 and 400 of the rat sequence. The cDNA was subcloned into the Blue Script plasmid vector (pBS, Stratagene), and both strands were sequenced by the dideoxy termination method as described (*24*).

turnover of this message (half-time <2 hours) in mammary tissue, since similar 3′ untranslated sequences are present in the rapidly turned over messages encoding a variety of cytokines and cellular oncogenes (*12*).

We examined the adenylate cyclase–stimulating activity of mammary tissue extracts in PTH-sensitive cells to determine whether PTH-LP mRNA is actually translated (*13*). This method had been used to detect and purify PTH-LP activity in tumors associated with HHM (*2–4*). Extracts from lactating tissue stimulated the production of ^{3}H-labeled adenosine 3′,5′-monophosphate (cAMP) in a dose-dependent manner (Table 1). This stimulation was inhibited by the PTH antagonist [D-Trp-12,Tyr-34]bovine PTH-(7–34)NH_2, indicating that the lactating mammary tissue actually produces a PTH-like activity.

Thus, the PTH-like peptide, initially characterized by its association with the clinical syndrome of HHM, is, in fact, produced by rat mammary tissue during lactation. Complementary DNA cloning reveals that the rat and human peptides are similar. The potent effects of this peptide on calcium metabolism (*8, 9*) and its production during lactation suggest that PTH-LP may act as a hormone that is produced by the mammary gland for the mobilization of bone calcium or its transfer to milk (or both). This possibility had previously been raised as an explanation for the bone loss during lactation (*14*). This hypothesis is also supported by the clinical observation of Rude *et al.* (*15*) that during lactation, normal serum calcium levels were maintained in a hypoparathyroid patient without the need for supplemental therapy. Determination of serum PTH-LP concentrations during lactation should corroborate this assumption.

The correlation between PTH-LP expression and lactation suggests a possible regulatory role for prolactin. The prolactin synthesis inhibitor bromocriptine was shown to block the large flux of calcium into milk and the related increase in calcium mobilization from the gut and the skeleton (*16–18*). Bromocriptine also reversed bone loss in hyperprolactinemic women (*19*). However, the mechanism of action of prolactin in the regulation of calcium homeostasis is not known (*18–20*). Since estrogen suppresses lactation (*21*), a possible relation between prolactin, estrogen, PTH-LP, and metabolic bone diseases such as postmenopausal osteoporosis deserves further investigation.

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