Origin of the Vitamin K-dependent Bone Protein Found in Plasma and Its Clearance by Kidney and Bone*

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The vitamin K-dependent bone protein (bone Gla protein, BGP) that is found in serum comes from new cellular synthesis rather than from the release of bone matrix protein during bone resorption. This is shown by the fact that Warfarin administration to normal rats completely shifts the chemical nature of serum BGP with time, from a form which has γ-carboxyglutamic acid to one which does not. Since the BGP isolated from the bones of rats 8 h after Warfarin injection is fully γ-carboxylated, the abnormal serum BGP could not have come from the resorption of bone matrix. The origin of serum BGP in new cellular synthesis is also supported by the fact that administration of vitamin K1 to a chronically Warfarin-treated rat causes serum levels of fully γ-carboxylated BGP to return to normal levels within 15 h. Since total BGP levels in these bones are only 1.5% of normal at 15 h after vitamin K administration, the normal BGP levels in serum cannot be due to the release of bone matrix BGP during bone resorption.

Injected BGP is cleared from the serum rapidly, with a half-time of 4 min. The primary mode of clearance is kidney filtration, as shown by the accumulation of 125I-label in the kidney within minutes of 125I-BGP injection. The importance of the kidney in the clearance of BGP from serum is also demonstrated by the fact that nephrectomy causes serum BGP levels to increase 8-fold in 4 h. Clearance of 125I-BGP by bone is quantitatively less important than is clearance by the kidney, accounting for only 7% of the injected label in 1-month-old rats. The accumulation of 125I-labeled BGP in bone, as well as the binding of BGP to hydroxyapatite, requires the presence of γ-carboxyglutamic acid in the protein. These results, together with other observations, support the hypothesis that the role of γ-carboxyglutamic acid in BGP is to enable the protein to bind strongly to hydroxyapatite and, by this association, accumulate in bone.

The vitamin K-dependent protein of bone is a 49-residue protein of known structure (1-3) which is found in the extracellular bone matrix (4, 5) and in blood plasma (6, 7). It contains three residues of the vitamin K-dependent amino acid, γ-carboxyglutamic acid, and has been termed bone Gla1 protein or Gla protein. BGP is synthesized by calf bone in culture (8) and by rat osteosarcoma cells with an osteoblastic phenotype (9, 10). It appears in calcifying tissues 1 to 2 weeks after mineral deposition and at the approximate time that the maturation of bone mineral to hydroxyapatite is thought to occur (7, 11). This evidence, together with the fact that BGP binds strongly to hydroxyapatite in an association which requires γ-carboxyglutamate (12, 13), has led to the suggestion that BGP is directed to bone by its affinity for hydroxyapatite (6).

Although the function of BGP in bone metabolism is not presently known, several lines of evidence suggest that this function may involve regulation of calcium or skeletal homeostasis, rather than the formation of bone structure. Rabbits maintained for up to two months on high dosages of Warfarin have normal bone morphology, strength, and mineral content in spite of BGP levels which are 5% of normal (13). In addition, rats maintained on Warfarin from birth fail to evidence any abnormalities in bone mineral content or structure, in spite of BGP levels which are 1 to 2% of normal (14). These experimental results are difficult to reconcile with the hypothesis that BGP plays a role in bone structure. An informational role for BGP is suggested by the fact that the molecular weight of BGP is only 5700, both as secreted by osteosarcoma cells (9) and as found in bone (1). An informational function for BGP is also supported by the discovery that the biosynthesis and secretion of BGP from osteosarcoma cells are regulated by physiological levels of 1,25(OH)2 vitamin D3 (15), a hormone which potently stimulates the mobilization of bone calcium (16).

If BGP has an informational function, the biologically active protein could be plasma BGP, bone matrix BGP, or BGP bound to bone mineral at its interface with plasma. To evaluate these possibilities, it is crucial to determine whether the BGP in plasma comes directly from new cellular synthesis or from the release of extracellular matrix BGP during bone resorption.

These two hypotheses for the origin of plasma BGP predict different outcomes for experiments in which the γ-carboxylation status of newly synthesized BGP is abruptly changed. If plasma BGP represents newly secreted protein, it should be possible to have a chemically different BGP in plasma than in the extracellular matrix of bone. If plasma BGP comes from bone matrix resorption, plasma BGP should always resemble BGP isolated from the bone of the same animal. In the present experiments, we have used Warfarin administration into normal rats, or vitamin K1 injection into chronically Warfarin-treated rats, to provide an abrupt change in the γ-carboxyglutamic acid to one which does not.

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The abbreviations used are: Gla, γ-carboxyglutamic acid; bone Gla protein (BGP), γ-carboxyglutamic acid-containing protein of bone; deCO2-BGP, BGP in which all 3 γ-carboxyglutamic acid residues have been thermally decarboxylated to glutamic acid. BGP has also been termed osteocalcin and the vitamin K-dependent protein of bone.
tion status of newly synthesized BGP. These experiments have shown that plasma BGP can be chemically different from bone matrix BGP and so must arise from new secretion and not from the release of BGP bone matrix during bone resorption.

The fact that plasma BGP arises from new synthesis suggests the possibility that bone matrix BGP may itself come from plasma BGP. Calculations based on the affinity of BGP for bone mineral and the concentration of BGP in plasma have in fact shown that the levels of BGP found in bone matrix are compatible with this model (6). In the present study, we have investigated the fate of plasma BGP by using injected, radioiodinated BGP to monitor clearance by kidney, bone, and other tissues.

**EXPERIMENTAL PROCEDURES**

**Materials**—Simonsen albino rats were purchased from Simonsen Laboratories, Gilroy, CA. Hydroxypatite (calcium phosphate tri-basic) was obtained from Mallinkrodt. Coumadin (sodium Warfarin) was purchased from Endo Laboratories, and aqueamphenytoin (vitamin K) from Merck Sharp & Dohme. The procedures for the purification of rat BGP, for the thermal decarboxylation of γ-carboxyglutamate residues in BGP, and for the iodination of BGP by the solid state lactoperoxidase method have been described elsewhere (7).

**Hydroxypatite Binding Assay**—A rapid and simple method was developed to determine quantitatively the relative amounts of γ-carboxylated and non-γ-carboxylated BGP in serum. This assay takes advantage of the previously observed difference in hydroxypatite binding of carboxylated and non-carboxylated BGP (13). In a typical assay, 100 μl of serum was added to a test tube (6 x 50 mm) containing 10 mg of hydroxypatite. The mixture was then vortexed, turned end-over-end at 4 °C for 30 min, and centrifuged for 5 min in a tabletop clinical centrifuge. The supernatant, which contains BGP unable to bind to hydroxypatite, was removed and saved for later radioimmunoassay (7). The remaining hydroxypatite pellet was washed twice with 500 μl of distilled H2O. Next, 500 μl of radioimmunomassay diluent (0.14 M NaCl, 0.01 M phosphate, 0.025 M EDTA, 0.1% gelatin, 0.1% Tween 20, pH 7.4) was added to the hydroxypatite pellet and the mixture was vortexed and turned end-over-end at 4 °C for 1 h. The mixture was then centrifuged as before and the supernatant, which contains all BGP previously bound to the hydroxypatite, was removed and saved for later radioimmunoassay (7).

**Warfarin Injection Studies**—The short term effects of Warfarin injection were studied in 1- and 10-month-old rats. Rats were injected subcutaneously with 7.7 mg of sodium Warfarin/100 g of body weight. Blood samples (500 μl) were removed by cardiac puncture at the appropriate times and serum was collected from blood samples allowed to coagulate overnight at 4 °C. Serum was assayed for γ-carboxylated and non-γ-carboxylated BGP by the hydroxypatite binding assay described above.

The effects of vitamin K, injection on serum BGP in Warfarin-maintained rats were studied in 18-day-old rats which had been maintained from birth on the Warfarin protocol (14). At time zero, all rats were given a subcutaneous injection of 10 mg of vitamin K. Rats were then exsanguinated by cardiac puncture at the appropriate times and serum was assayed for γ-carboxylated and non-γ-carboxylated BGP by the hydroxypatite binding assay.

**Clearance of Injected Rat BGP**—Rats weighing 100 g were fasted overnight prior to injection. Approximately 150 μg of pure rat BGP in phosphate-buffered saline containing 0.4% ovalbumin was injected into the jugular vein. Control rats were injected with carrier alone. A series of 0.3-ml blood samples were removed from the jugular vein at the appropriate times and the serum BGP levels were determined by radioimmunoassay.

**Clearance of 125I-labeled BGP**—In short term studies, rats (300 g) were injected in the jugular vein with 2 x 10⁵ cpm per 100 g of body weight of 125I-labeled rat BGP in radioimmunoassay diluent (7). Rats were bled out via the abdominal aorta and immediately dissected. Samples of various tissues were weighed and assayed for the level of 125I label in a gamma counter.

Long term studies were done in 1- and 10-month-old rats. Rats were injected in the jugular vein with either iodinated native BGP or iodinated thermally decarboxylated BGP at a dosage of 2 x 10⁵ cpm/100 g of body weight. The level of 125I label in the intact rat was measured within 5 min of injection and on each subsequent day in a J.L. Shepherd and Associates model 209 gamma counter. All determinations were corrected for decay of the 125I isotope. After 7 days, the rats were killed and the level of 125I label in intact organs was measured in the model 209 gamma counter.

**Nephrectomy Experiments**—Rats weighing 300 g were nephrectomized by ligating the renal artery and vein of each kidney and then removing the organ. In sham-operated rats the kidneys were exposed identically but not removed. Serial blood samples (500 μl) were removed by cardiac puncture and BGP levels in serum were determined by radioimmunoassay.

**RESULTS**

**Binding of Serum BGP to Hydroxypatite**—In previous investigations, we have shown that native BGP binds to hydroxypatite more strongly than BGP in which all three γ-carboxyglutamic acid residues have been thermally decarboxylated to glutamic acid (12, 13). Since Warfarin administration should produce a non-γ-carboxylated BGP analogous to thermally decarboxylated BGP, hydroxypatite binding could provide a convenient means to discriminate between normal and non-γ-carboxylated BGP in serum. To evaluate this possibility, purified preparations of native rat BGP and of thermally decarboxylated rat BGP were titrated from calf serum by the addition of increasing amounts of hydroxypatite. The amount of BGP not bound to hydroxypatite was then determined by radioimmunoassay of the supernatant, a procedure which, because of antibody specificity, permits the detection of both native and thermally decarboxylated rat BGP in the presence of calf serum BGP (7). As can be seen in Fig. 1, hydroxypatite binding does provide a means by which to discriminate between native and decarboxylated BGP in serum.

![Binding of purified rat BGP to hydroxypatite](image-url)
Source of the Vitamin K-dependent Bone Protein in Plasma

In order to see if Warfarin treatment results in a serum BGP which resembles thermally decarboxylated BGP in its inability to bind to hydroxyapatite, we next compared the serum BGP from normal rats with that from chronically Warfarin-treated rats. The Warfarin-treated rats used in this study had 1% of normal BGP levels in bone and somewhat elevated BGP levels in serum (14). As can be seen in Fig. 2, the serum BGP from the chronically Warfarin-treated rat has the same inability to bind to hydroxyapatite, as does thermally decarboxylated BGP. In addition, serum BGP from a control rat resembles BGP purified from rat bone in its ability to strongly bind to hydroxyapatite. These results strongly suggest that the serum BGP in Warfarin-maintained rats is not $\gamma$-carboxylated. It should be noted, however, that partially $\gamma$-carboxylated BGP species may be present which cannot bind to hydroxyapatite and so are counted as non-$\gamma$-carboxylated.

The presence of non-$\gamma$-carboxylated BGP in the serum of Warfarin-maintained rats is also supported by the fact that serum BGP from Warfarin-maintained rats has the same Sephadex G-100 elution position and the same electrophoretic mobility in 20% polyacrylamide gels as thermally decarboxylated BGP (data not shown) (see Ref. 12 for procedures).

Effect of Warfarin on Serum BGP in Normal Rats—As can be seen in Fig. 3, Warfarin administration to a 1-month-old rat produces a rapid, 3-fold increase in total serum BGP levels. Fig. 4 shows that this increase is due to a rise in the fraction of BGP which does not bind to hydroxyapatite. The fraction of serum BGP that can bind hydroxyapatite declines from 95 to 10% of the total by 4 h. Although the administration of Warfarin to a 10-month-old adult rat does not change total serum BGP levels (Fig. 3), Fig. 5 shows that it does change the ability of serum BGP to bind to hydroxyapatite. Within 4 h of Warfarin administration to adult rats, there is a dramatic shift from a serum BGP which is primarily bound to hydroxyapatite to one which is primarily not bound to hydroxyapatite. These studies demonstrate that Warfarin ad-

Fig. 2. Binding of rat serum BGP to hydroxyapatite. Rats were maintained from birth to day 18 on the Warfarin protocol or to day 29 on the control protocol as described (14). Aliquots of sera from control (○) and from Warfarin-maintained (●) rats were mixed with the indicated quantities of hydroxyapatite for 30 min at 4 °C and the level of protein remaining in solution was determined by triplicate radioimmunoassay.

Fig. 3. Effect of Warfarin on serum BGP levels in 1- and 10-month-old rats. At time zero, all rats received 7.7 mg of Warfarin/100 g of body weight. Serum BGP levels were determined by triplicate radioimmunoassay of 10-μl serum samples. Each data point is the average serum BGP level in three rats bled at the indicated time. ●, 1-month-old rats; ○, 10-month-old rats.

Fig. 4. Effect of Warfarin on hydroxyapatite binding properties of serum BGP in 1-month-old rats. Serum samples from Fig. 3 were tested by the hydroxyapatite binding assay as described under "Experimental Procedures." ●, serum BGP not bound to hydroxyapatite; ○, serum BGP bound to hydroxyapatite.
administration rapidly changes the apparent γ-carboxylation status of serum BGP in both young and adult rats. The effect of short term Warfarin treatment on the nature of bone matrix BGP was evaluated using BGP isolated from the bones of rats killed at the end of these experiments, 8 h after Warfarin administration. Amino acid analysis of this purified bone matrix BGP showed that it has a normal complement of 3 γ-carboxyglutamate residues and hydroxyapatite binding studies demonstrated that it binds to bone mineral normally. Thus, the abnormal BGP found in serum following Warfarin administration could not have come from the release of an abnormal bone matrix BGP during bone resorption. The serum BGP in Warfarin treated animals must instead arise from new cellular synthesis and its secretion directly into serum.

It seems probable that the rise in total serum BGP levels following Warfarin administration to young rats is due to the appearance in serum of that fraction of new BGP synthesis which normally accumulates in the mineral phase of bone. The 3-fold rise in serum BGP following Warfarin administration would then indicate that at least 75% of new BGP synthesis in young rats is normally deposited in bone rather than in serum. The fact that total serum BGP levels do not rise following Warfarin administration to adult rats would be explained by the fact that the rate of bone growth is far slower in adult rats and so the fraction of new cellular secretion which normally accumulates in adult bone is insignificant compared to the fraction which is secreted into serum.

Effect of Vitamin K, on Serum BGP in Chronically Warfarin-treated Rats—The administration of vitamin K₁ to a chronically Warfarin-treated rat provides a second test for the origin of serum BGP. The serum BGP in these rats is initially abnormal and cannot bind to hydroxyapatite (Fig. 2). After the administration of vitamin K₁ to these rats, there is a rapid appearance of a BGP species in serum which will bind to hydroxyapatite (Fig. 6). By 15 h, the level of serum BGP bound to hydroxyapatite has reached the level in control animals. Since the BGP level in the bone matrix of these rats is still only 1.5% of the control level at this time (Fig. 7), we can conclude that the return to normal levels of plasma BGP bound to hydroxyapatite reflects new cellular synthesis and its secretion into plasma.

The accumulation of BGP in the bone of chronically Warfarin-treated rats following vitamin K₁ injection is compared with the appearance of normal BGP in serum in Fig. 7. Clearly, the level of serum BGP bound to hydroxyapatite reaches normal values at about the time that BGP begins to accumulate in bone matrix. These results are consistent with a model in which BGP is directed to bone matrix by its affinity for hydroxyapatite. Each unit of new bone hydroxyapatite is presumably in contact with the pool of newly synthesized BGP for a brief period before it is permanently sequestered. If newly synthesized BGP is not γ-carboxylated, no BGP accumulates and we observe the low BGP levels in bone matrix BGP.
Source of the Vitamin K-dependent Bone Protein in Plasma

Fig. 8. Clearance of injected rat BGP from serum. Rats (100 g) were given intrajugular injections of 150 μg of purified rat BGP. Each point represents the average serum BGP level in 3 animals bled at that time with the exception of the 6 and 10 h points, which were 1 animal each.

Table I

<table>
<thead>
<tr>
<th>Tissue fraction</th>
<th>Relative 125I label in tissue fractions at indicated minutes after 125I-BGP injection</th>
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<tbody>
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<td></td>
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<tr>
<td>Liver</td>
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</tr>
<tr>
<td>Spleen</td>
<td>1.6</td>
</tr>
<tr>
<td>Bone</td>
<td>0.9</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.3</td>
</tr>
<tr>
<td>Fat</td>
<td>0.9</td>
</tr>
<tr>
<td>Serum</td>
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<tr>
<td>Urine</td>
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<tr>
<td>Kidney, whole</td>
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<td>Medulla</td>
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</tr>
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<td>Cortex</td>
<td>17</td>
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matrix found in the Warfarin-treated rat. If newly synthesized BGP is fully γ-carboxylated, as after vitamin K1 injection, bone BGP levels slowly rise as each unit of newly formed hydroxyapatite now binds a normal complement of BGP, the result seen in Fig. 7.

Clearance of BGP from Serum by the Kidney—Several experiments indicate that BGP is cleared rapidly from serum. The injection of purified rat BGP into a rat produces a transient elevation in plasma BGP which returns to the normal level in 5 h (Fig. 8). The time needed to clear half of the administered BGP from serum is less than 5 min. Similar results are also obtained by following the rate of clearance of iodinated rat and calf BGP from serum (data not shown).

To determine the probable mode of BGP clearance, rats were killed at suitable times after the administration of radioiodinated rat BGP and the tissue distribution of radioactivity was determined. Most of the administered radioactivity was recovered in the kidney (Table I), which identifies clearance by the kidney as the probable major clearance mechanism for serum BGP. It should be noted that rapid clearance of serum protein by kidney filtration has been observed with small proteins such as RNase (17) and so would be the expected mode of clearance for the 5700-dalton BGP.

If BGP is normally cleared rapidly from serum by the kidney, the actual BGP level in serum must represent a dynamic balance between the rates of new synthesis and kidney clearance. Nephrectomy should therefore block clear-

Fig. 9. Effect of nephrectomy on levels of BGP in serum. Rats (300 g) were nephrectomized or sham-operated at time zero as described under “Experimental Procedures.” Each point is the serum BGP level in one rat as determined by triplicate radioimmunossay.

Fig. 10. Clearance of 125I from rats injected intrajugularly with 125I-labeled BGP. Whole body counts were obtained on animals at suitable times after injection with 125I-labeled native BGP (●), or 125I-labeled, thermally decarboxylated BGP (○), as described under “Experimental Procedures.” Each point is the per cent of administered label retained in one animal; all points have been corrected for the decay of 125I. A, 10-month-old rats; B, 1-month-old rats.
ance and cause a rapid increase in serum BGP levels. Fig. 9 shows that this is indeed the case. Serum BGP levels double 20 min after nephrectomy and reach 8 times the normal level in 4 h. The serum BGP levels continue to increase after 4 h and, by 24 h, are 15 times the normal level (data not shown).

**Clearance of BGP from Serum by Bone**—To evaluate the importance of bone in the clearance of BGP from serum, rats were injected intravenously with iodinated BGP and the long term fate of the label was determined. As can be seen in Fig. 10, in both young and old rats, over 75% of the administered label is excreted in 2 days. This result is consistent with the identification of kidney filtration as the major mode of serum BGP clearance. The label remaining after 2 days is removed from the rat more slowly with a half-time of 6 days. Since negligible label remains in serum by 2 days, the labeling in the animal must have entered organs where its turnover is slow. The organ distribution of the label remaining in rats 7 days after the administration of BGP is shown in Table II. There is significant accumulation of the native BGP label in the skulls and long bones of both young and old rats. This accumulation depends on the presence of γ-carboxyglutamate residues, as shown by the dramatically lower accumulation of thermally decarboxylated BGP in these bones. Other organs also accumulate significant levels of label, particularly the thyroid and kidney. The high levels of 125I-label in the thyroid probably reflect the degradation of 125I-labeled BGP and the reincorporation of labeled iodide into thyroglobulin.

The quantitative importance of bone in the clearance of serum BGP can be evaluated if it is assumed that native BGP accumulates in bone while non-γ-carboxylated BGP does not. Since equal amounts of labeled native and thermally decarboxylated BGP are found in the older rats at day 7 (Fig. 10), bone is not a significant clearance mechanism for serum BGP in these animals. In contrast, the younger rats do retain more label at 7 days when injected with native BGP than when injected with thermally decarboxylated BGP. If the higher retained label is due solely to the accumulation of native BGP in bone, ~7% of the label of the administered BGP has been cleared by bone.

<table>
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<th>Tissue fraction</th>
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<th>10-month-old rat</th>
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<td><strong>125I-deCO2</strong></td>
<td><strong>125I-BGP</strong></td>
</tr>
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<td>Liver</td>
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<tr>
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<td>12.8</td>
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<tr>
<td>Intestines</td>
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<td>2.9</td>
</tr>
<tr>
<td>Lung</td>
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<td>0.2</td>
</tr>
<tr>
<td>Heart</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Brain</td>
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<td>0.1</td>
</tr>
<tr>
<td>Skin</td>
<td>2.8</td>
<td>2.2</td>
</tr>
<tr>
<td>Skull</td>
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</tr>
<tr>
<td>Long bones</td>
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<tr>
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</tr>
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<td>Upper torso</td>
<td>24.3</td>
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</table>

**DISCUSSION**

The present demonstration that plasma BGP arises from new cellular synthesis indicates that plasma BGP measurements directly reflect the underlying rate of BGP synthesis rather than the rate of bone matrix resorption. This conclusion clarifies the interpretation of several previous results. The fact that 1,25-dihydroxyvitamin D₃ produces a 3-fold increase in plasma BGP levels (20) can now be taken as direct evidence that vitamin D regulates BGP synthesis in rats. The demonstration (21) that dietary Ca²⁺ deficiency also causes a 3-fold increase in plasma BGP in rats, and that dietary vitamin D deficiency abolishes this increase, can now be interpreted as direct evidence that BGP synthesis is regulated by vitamin D in the physiological response to Ca²⁺ deficiency in rats. Finally, the observation that plasma BGP levels are elevated in patients with metabolic bone diseases characterized by increased bone turnover now provides evidence that the rate of BGP synthesis is elevated in these disease states.

It is worth noting that there may be other explanations for the increased plasma BGP levels seen in these disease states. For example, it is possible that a disease state could interfere with the deposition of new BGP into bone matrix and so add increment of new synthesis to plasma. However, the results of the present experiments with Warfarin-treated adult rats (Fig. 3) clearly show that complete inhibition of BGP binding to bone produces no detectable increase in plasma BGP levels in adults. In other studies, we have found that Warfarin administration to human adults also has no effect on circulating levels of BGP. Another possible explanation for the increased serum BGP levels is that bone matrix BGP could, in some disease states, be released during bone resorption in an immunoreactive form and so add to plasma BGP levels. While this possibility cannot be ruled out presently, it is difficult to imagine osteoclastic bone resorption without the involvement of proteases and, given the sensitivity of the BGP immunoreactivity to widely different proteases (6), it seems unlikely that bone matrix BGP can selectively escape proteolytic digestion. Finally, it is possible that plasma BGP levels can be elevated in some metabolic bone disease states because the clearance of plasma BGP by the kidneys has been impaired. Plasma levels of BGP are, in fact, elevated dramatically in patients with renal osteodystrophy (19). Since we have shown here that nephrectomy in rats causes a 15-fold elevation in plasma BGP levels within 24 h, it seems likely that a major component of the increase in plasma BGP observed in patients with renal osteodystrophy is caused by impaired BGP clearance from serum, rather than from increased BGP synthesis.

While the present experiments demonstrate that some plasma BGP does accumulate in bone, a simple calculation shows that the fraction of 125I-labeled BGP deposited in the bone of young rats is too low for plasma BGP to be the sole source of bone matrix BGP.² It is possible that we have underestimated the importance of bone in the clearance of serum BGP because 125I-labeled BGP binds to bone less well than unlabeled BGP. However, it seems probable that BGP

² A 1-month-old rat weighing 85 g has ~5 ml of plasma; with 250 ng of BGP/ml of plasma (Fig. 3), 1.25 μg of BGP are in circulation. If half of this is cleared every 5 min (Fig. 8), 180 μg of BGP are removed from plasma every day. Since 7% of the BGP cleared from plasma goes to bone, bone must receive 12 μg BGP/day from plasma. At 1 month of age, our control rats gain 3.5 g/day (14). If we estimate that 10% of their weight is bone, they must make 0.35 g of bone/day. Since rat bone contains 3 mg of BGP/g (7), 1.05 mg of BGP must be deposited in bone/day. This is 80-fold greater than the 12.6 μg of 125I-BGP which we find experimentally to be deposited in bone each day.
accumulates in bone by its affinity for hydroxyapatite and we have shown here and elsewhere (12) that $^{251}$T-labeled and native BGP bind to hydroxyapatite with equal affinity. Another possibility is that most BGP in the extracellular matrix of bone is derived from cell secretion near newly formed hydroxyapatite. Serum BGP would then represent that fraction of newly synthesized BGP which fails to anchor in bone before it diffuses into plasma. While there is insufficient evidence to decide which of these two explanations for the low accumulation of $^{251}$T-labeled BGP in bone is correct, the second seems more likely based on present evidence.

We have previously suggested that the role of $\gamma$-carboxyglutamic acid residues in BGP is to enable the protein to bind to hydroxyapatite and, by this interaction, to accumulate in bone (1). The present results support this hypothesis strongly. Purified BGP in which all $\gamma$-carboxyglutamic acid residues have been thermally decarboxylated to glutamic acid has a far lower ability to bind from plasma to hydroxyapatite (Fig. 1) or to bone (Table II) than does native BGP. In addition, Warfarin treatment, which inhibits the $\gamma$-carboxylation of glutamic acid residues in vitamin K-dependent proteins (18), produces a plasma BGP which has a far lower ability to bind from plasma to hydroxyapatite than native BGP (Fig. 2). In other studies, we have shown that chronic Warfarin treatment also produces a 50-fold reduction in bone BGP levels (14). Finally, in several calcifying systems, it has been shown that BGP appears 1 to 2 weeks after the initial appearance of mineral. A likely explanation (7, 11) for the delay in BGP appearance is that it accumulates in bone only after the mineral phase has matured to hydroxyapatite, the mineral phase which binds BGP strongly. Thus, it seems probable that the role of $\gamma$-carboxyglutamic acid in BGP is to enable the protein to bind hydroxyapatite strongly, and by this interaction, accumulate in bone.

The present results can be interpreted in terms of an informational model for BGP function. In this model, BGP binds to exposed hydroxyapatite crystals in bone to an extent which depends critically on the concentration of BGP in the surrounding solution. The density of BGP on bone crystals then provides a signal which regulates osteoblastic or osteoclastic cellular activity in bone. Evidence in support of a regulatory function for BGP is provided by the recent discovery that the synthesis of BGP is regulated by 1,25-dihydroxyvitamin D$_3$, both in cell culture (15) and in rats (20, 21). Since BGP is the first bone-specific, secreted protein regulated by 1,25-dihydroxyvitamin D$_3$, it seems reasonable to propose that BGP may mediate the action of vitamin D in mobilizing bone Ca$^{2+}$ and that it acts by virtue of its ability to bind to hydroxyapatite in bone.

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REFERENCES