



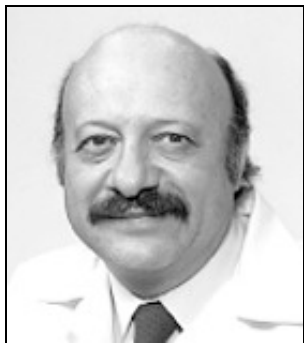
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## American Society of Nephrology Satellite Symposium on Hyperphosphatemia -- November, 1999

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# Role of Phosphate in the Pathogenesis of Secondary Hyperparathyroidism

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**Dr. Slatopolsky**

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#### **Dr. Bart W. Galle, Jr.:**

It is my pleasure to actually get the program started, to introduce our first speaker, Dr. Eduardo Slatopolsky, Professor of Renal Disease and Medicine at Washington University in St. Louis. We had the pleasure of working with Dr. Slatopolsky on a [similar program last year](#), and as many of you know, he received this morning the Scribner Award for his seminal work in secondary hyperparathyroidism and subsequent work in related fields. It's a great pleasure to have him here as our first speaker. His topic is the role of phosphate in the pathogenesis of secondary hyperparathyroidism. Please welcome Doctor Eduardo Slatopolsky.

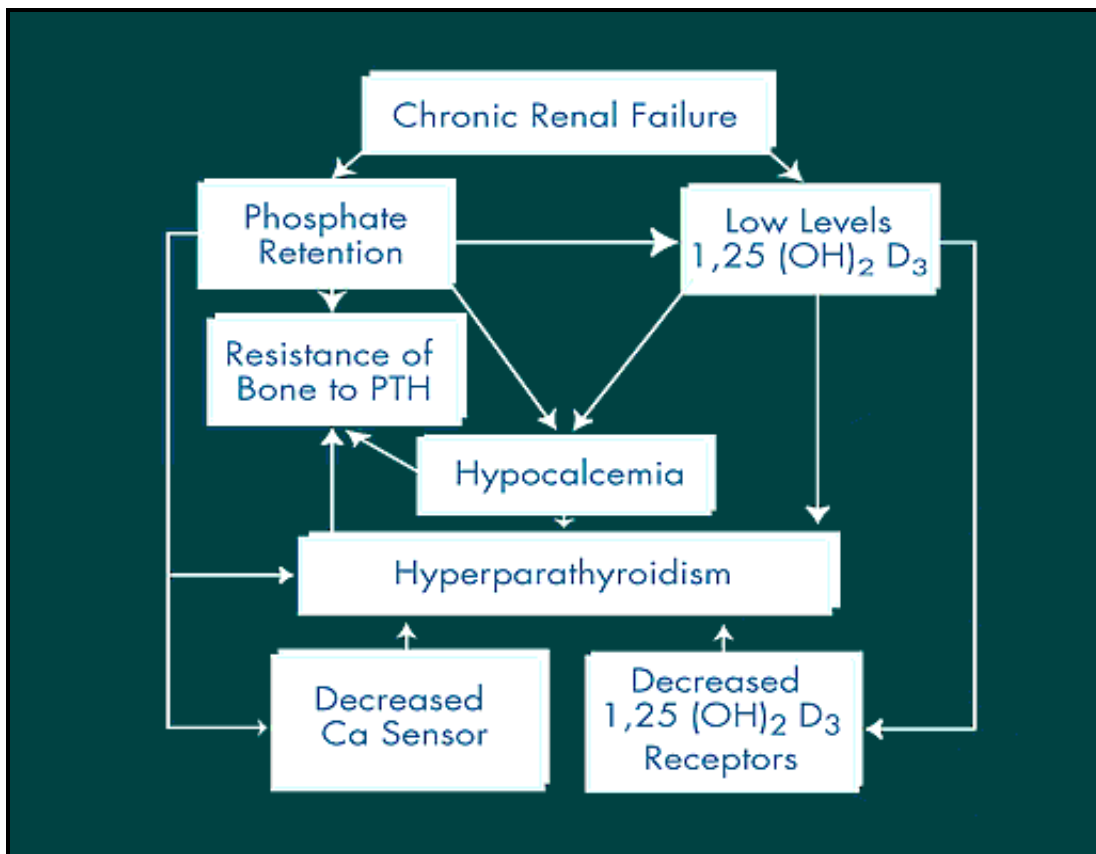
#### **Eduardo Slatopolsky:**

Thank you very much for coming tonight. I know it has been a very, very long day for all of

us since 7:30 this morning. I really appreciate your presence tonight. What we are trying to do tonight is to see what hyperphosphatemia does to our patients with renal insufficiency. And as you heard, we have three speakers.

My task tonight is to emphasize the role of phosphorus in the pathogenesis of secondary hyperparathyroidism. And other speakers will continue with other significant advances in the management of hyperphosphatemia.

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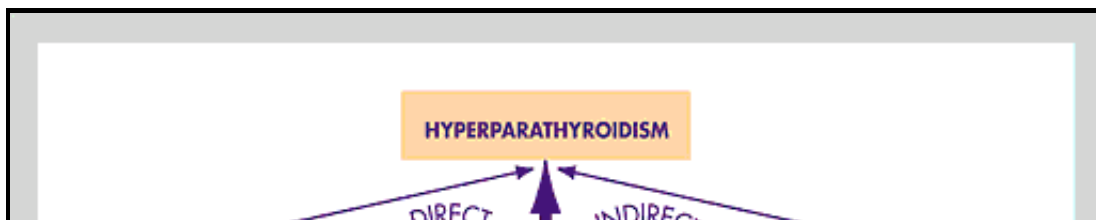


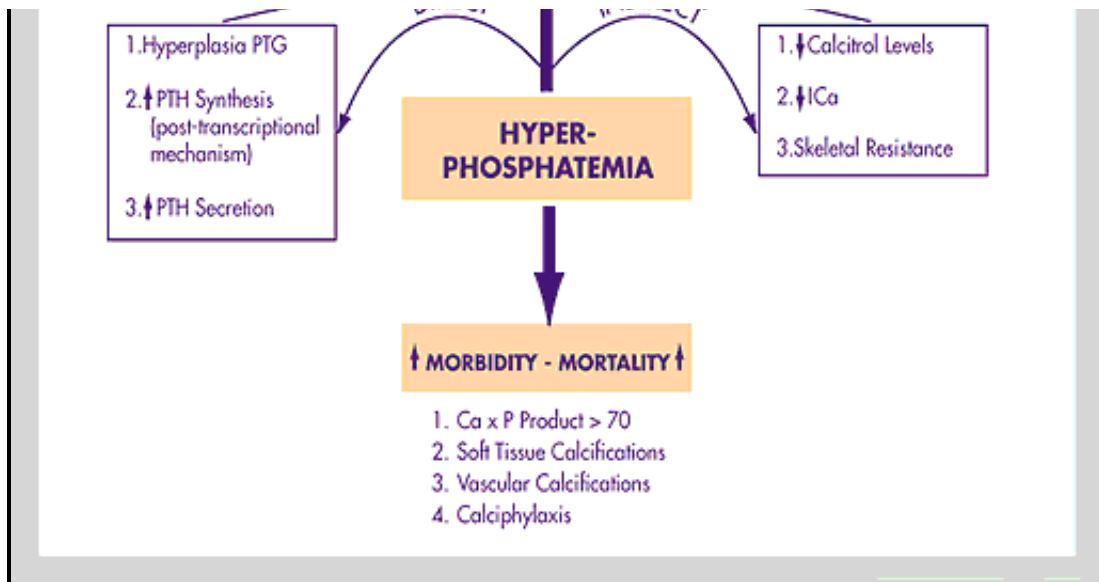
### How chronic renal failure causes hyperparathyroidism

As you know, the great majority of patients with renal failure develop secondary hyperparathyroidism. And now we finally know that there are three important players.

- We knew for many, many years that calcium, hypocalcemia triggered the release of PTH.
- We learned in the '70's that low levels of calcitriol decreased the absorption of calcium and induced hyperparathyroidism,
- but we learned later on that calcitriol, per se, independent of calcium suppressed pre-pro PTH mRNA.
- Also, we learned in the '80's that the number of Vitamin D receptors are very low in renal insufficiency, therefore it's resistant to calcitriol because of a low number of receptors.

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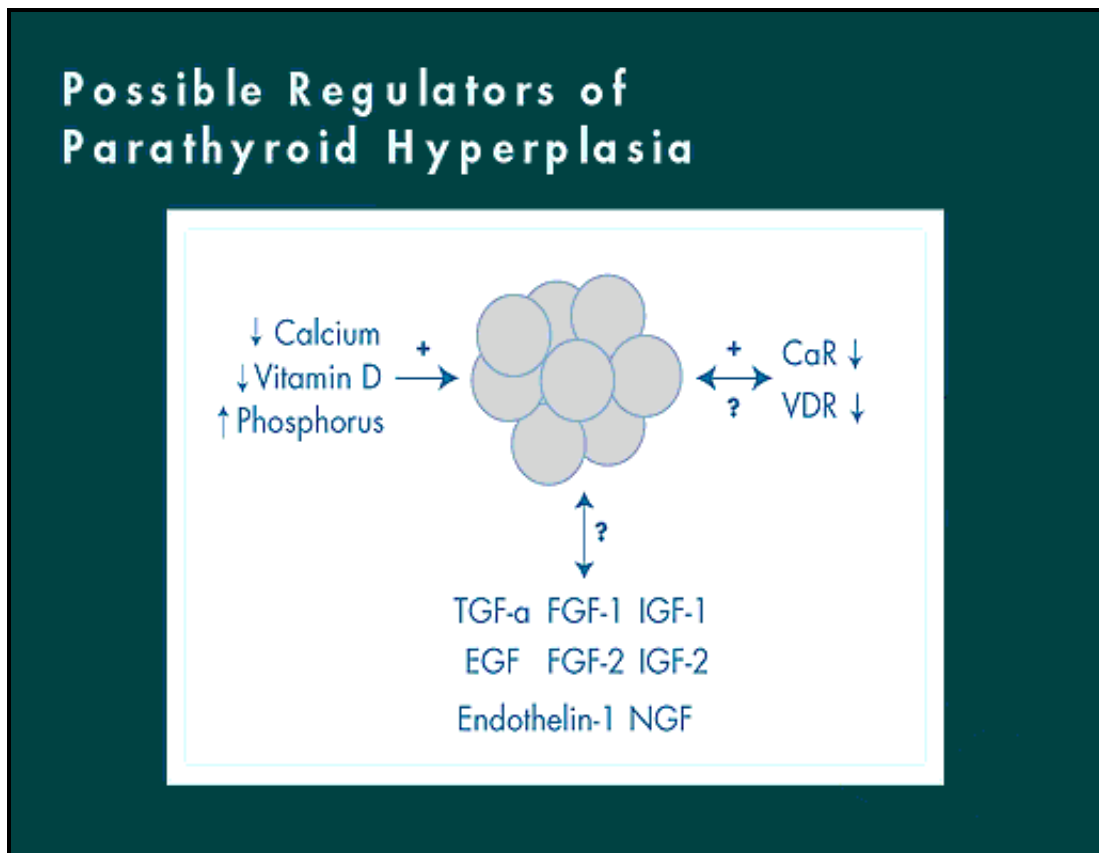


### Specific role for phosphate

We learned many years ago that phosphate can induce hyperparathyroidism by decreasing the levels of 1,25 or by inducing hypocalcemia. But we and others have shown in the past five years that phosphate, per se, independent of calcitriol, independent of calcium, has a direct effect on the secretion of parathyroid hormone.

My role tonight is to concentrate on this part, in the so-called phosphate, and since tonight we are going to have another symposium with another pharmaceutical company, tomorrow we're going to do the vitamin D part. Then if you come to both of them, you get the whole picture. But tonight I'm supposed to talk about this part of this scheme.

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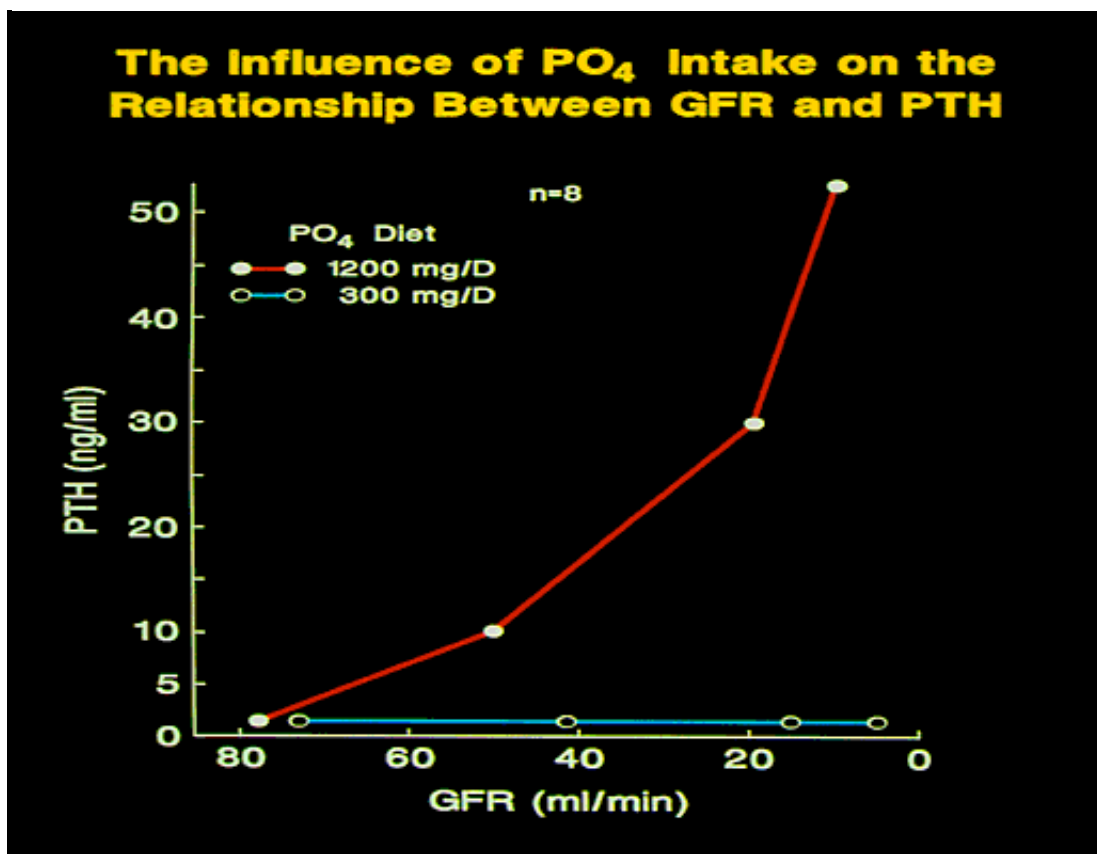
Importance of parathyroid gland hyperplasia in uremia

It's not only important to control hyperparathyroidism, but to control the growth of the parathyroid cell. When a patient or an animal develops chief cell hyperplasia, it's almost impossible to bring the cells back to normal. We may reduce the volume, but not the hyperplasia. And there is no apoptosis. That is, when a patient has very large glands, although we may have seen some changes in the size of parathyroid gland, the change is very small, and the change in DNA is practically zero. That is critical, the prevention. Since we know that low calcium and low vitamin D, or high phosphorus will induce hyperplasia, and we are studying the effect of the receptors, and some growth factors are important.

### **Role for phosphate via TGF in causing parathyroid gland hyperplasia**

We know now, for the first time, that TGF-alpha is induced by hyperphosphatemia and increases the cells in the parathyroid gland. Those of you who would like to know a little more about TGF-alpha it will be present in great detail by one of our collaborators, Dr. Adriana Dusso, tomorrow in the 4:00 to 6:00 pm meeting in PTH and PTHrP (Dusso AS et al, A role for enhanced expression of transforming growth factor-alpha (TGF- alpha) in the mitogenic effect of high dietary phosphorus on parathyroid cell growth in uremia. J Am Soc Nephrol (abstract) 10:617A, 1999).

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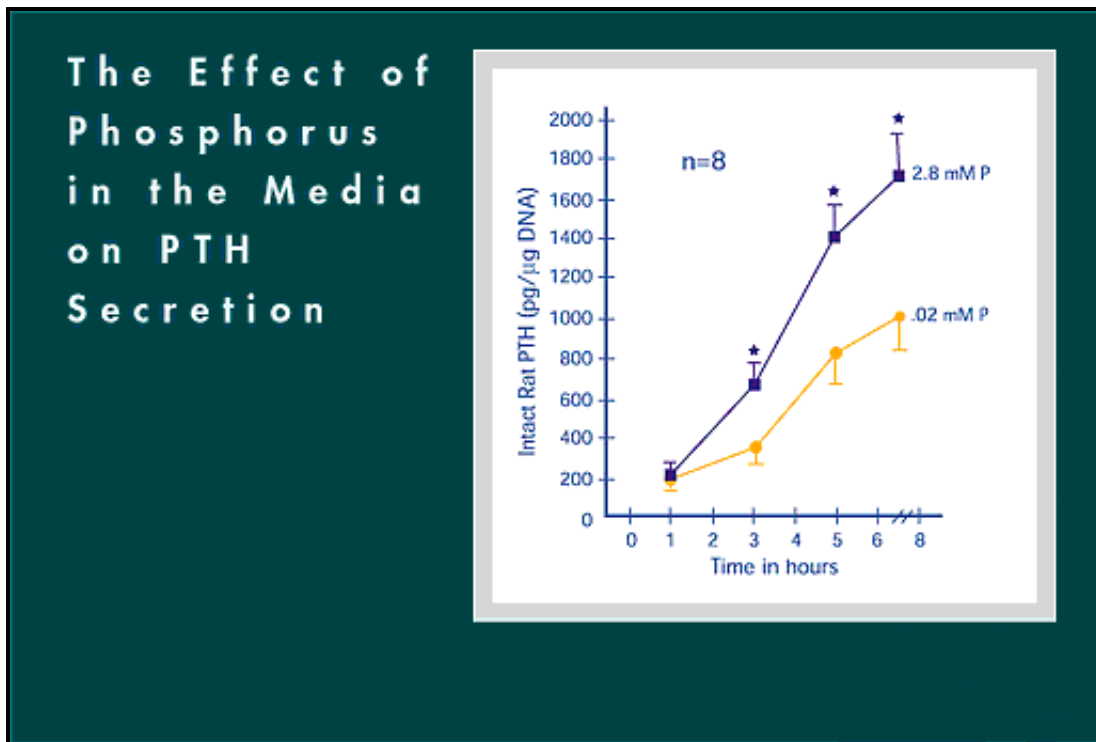


*Source: Slatopolsky E et al, Kidney Int 1972 Sep;2(3):147-51.*

### **High phosphate diet in uremic dogs induces, and low phosphate diet prevents, hyperparathyroidism**

This slide is about 35 years old, and was shown this morning by my good friend, Dr. S. Klahr, and was the first demonstration that if you induce renal failure in dogs, and you feed them a normal phosphate intake, they develop hyperparathyroidism.

On the other hand, if you restrict phosphorus in the diet, in blue, they did not develop hyperparathyroidism. Remember, this was the late '60's, and we didn't know anything about calcitriol, we didn't know anything about receptors. But that was the first indication that phosphorus was doing something to the parathyroid glands. I'm going to go past 75 slides, otherwise it will take me the whole night, next slide please, and I'm going to show the results from the past two or three years.



*Source: Slatopolsky E et al, J Clin Invest 1996 Jun 1;97(11):2534-40.*

### Phosphorus enrichment of culture media induces increased PTH synthesis by cultured parathyroid glands

What we have shown two or three years ago, that if you incubate parathyroid glands from normal rats and you increase the amount of phosphorus in the culture medium from 0.2 to 2.8 mM and for those of you who are accustomed to mg/dl, you have to multiply by 3.1, as you increase the amount of phosphorus in the culture medium, there is a significant increase in the release of PTH. The levels of calcium and calcitriol in the culture medium were identical.

Other investigators, Moreno Rodriguez and his group, Dr. Silver in Jerusalem and his group, and several others have shown the same results. Finally, after 35 years, there is agreement between investigators, that phosphorus, per se, produces secondary hyperparathyroidism.

## Methodology

### Diet

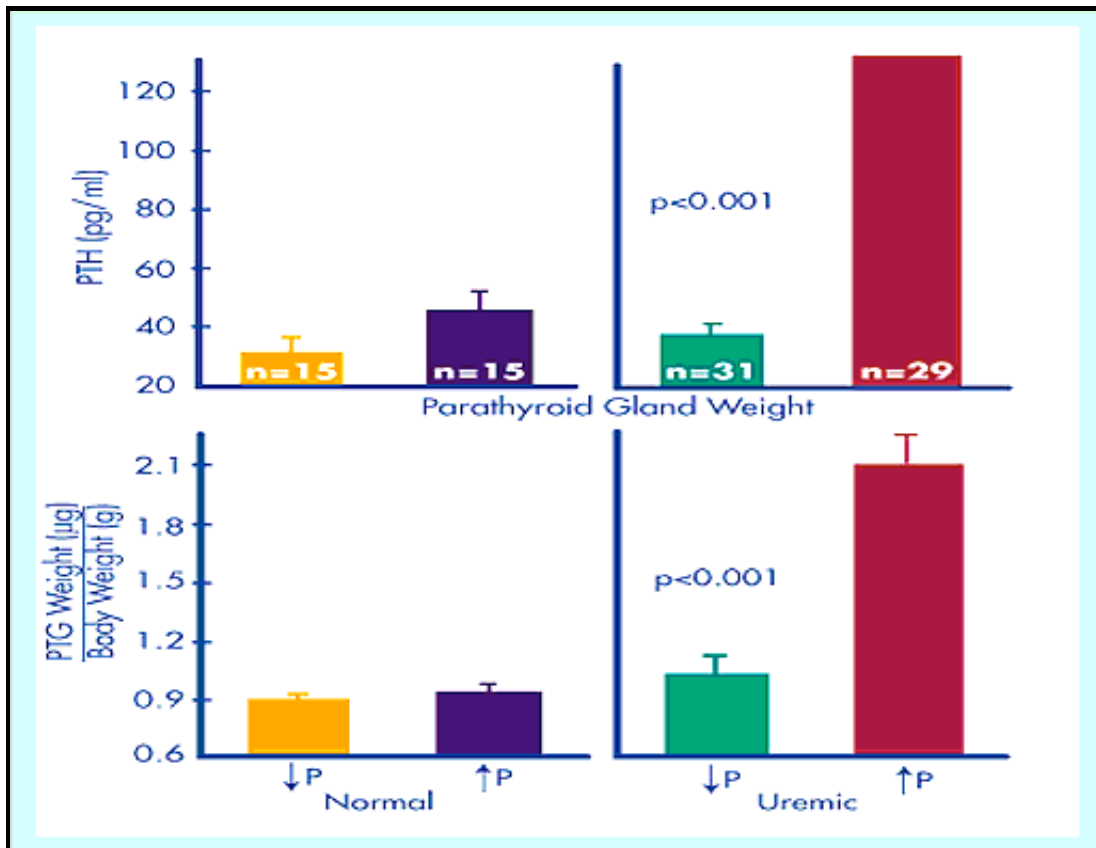
	% Ca	% P
I. Normal	0.5	0.2
II. Uremic	0.5	0.2
III. Normal	1.0	0.8

Source: Slatopolsky E et al, J Clin Invest 1996 Jun 1;97(11):2534-40.

### Effects of high vs low phosphorus diet in uremic rats on parathyroid gland size

What about chief cell hyperplasia? Can phosphorus also increase the size of the parathyroid glands? What we did is the following experiment. We took normal rats and uremic rats, and we fed them a low phosphorus diet, 0.2 mM, or a high phosphorus diet, 0.8 mM. And the results are depicted in the next slide.

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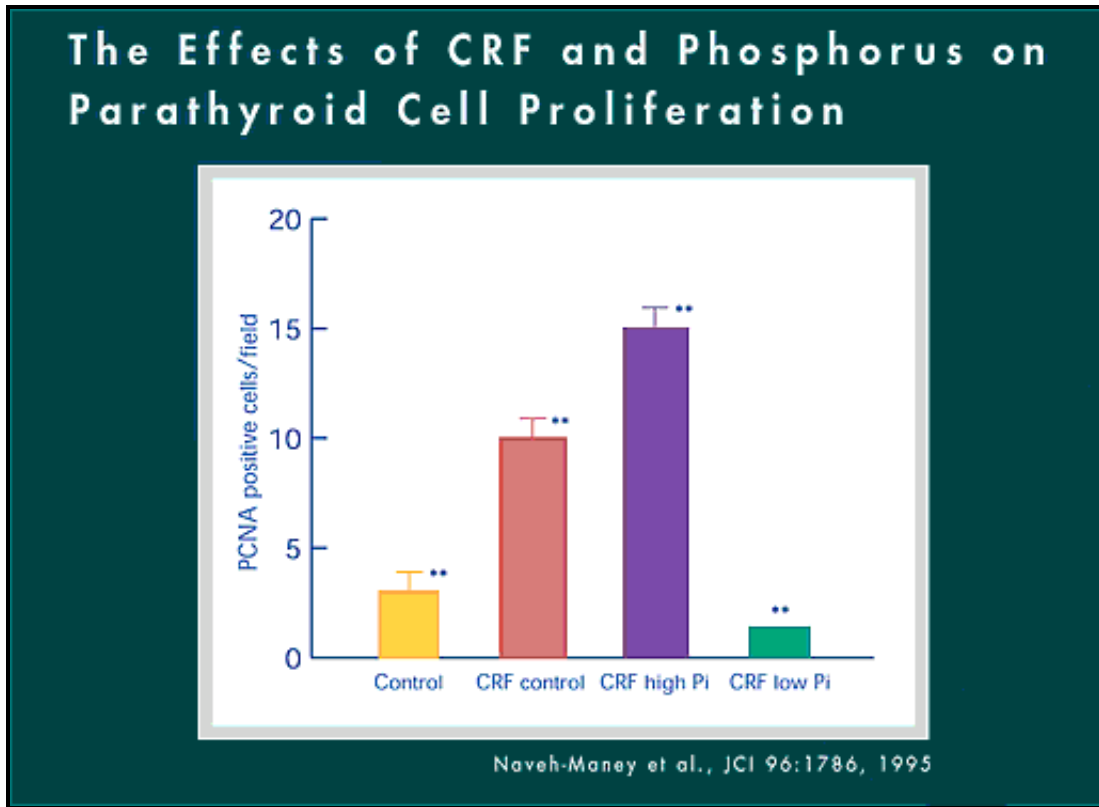
Source: Slatopolsky E et al, J Clin Invest 1996 Jun 1;97(11):2534-40.

### Parathyroid gland weight is increased only in uremic rats on the high phosphorus diet

In blue, I have depicted the normal rats, and in red, you may not be able to see it very well, are uremic rats. If you can please concentrate here, these are the uremic rats who received the low phosphorus diet. And here we have PTH. As you can see, they did not develop secondary hyperparathyroidism. The level of PTH is the same like normal animals.

On the other hand, the uremic animals, on a high phosphorus diet, PTH increased from 30 to 120. These animals developed secondary hyperparathyroidism.

If you think for a minute, I have not told you anything new, that I didn't tell you 35 years ago. Because we showed that, in dogs, when we restricted the diet. But at that time, we didn't measure the size of the parathyroid glands, we didn't measure protein and DNA, and we have done it now, and those animals on a low phosphorus diet, the size of the gland, DNA, and protein is the same like normal. On the other hand, on a high phosphorus diet, they develop chief cell hyperplasia. Thus, not only did we induce hyperplasia, but also we get hyperparathyroidism, independent of calcium, and independent of calcitriol.

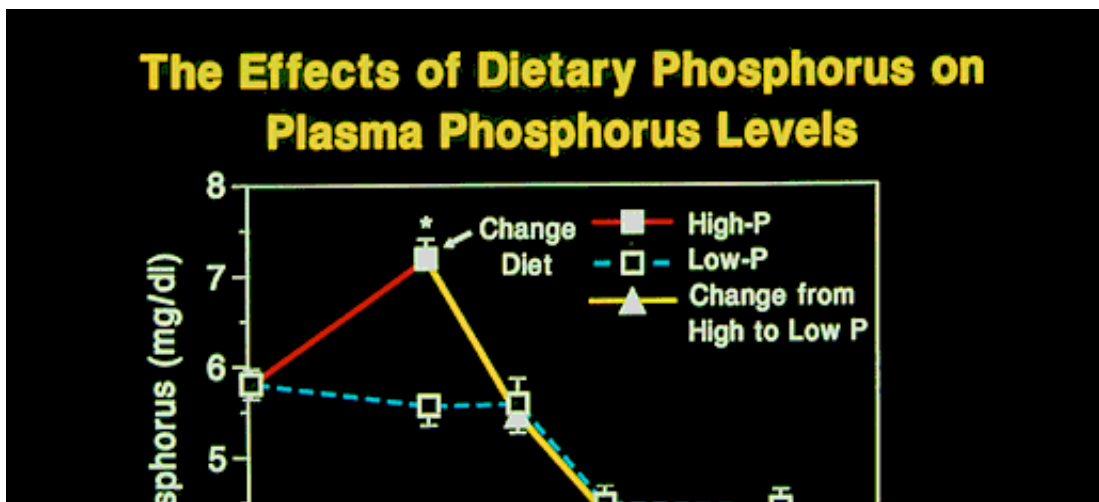


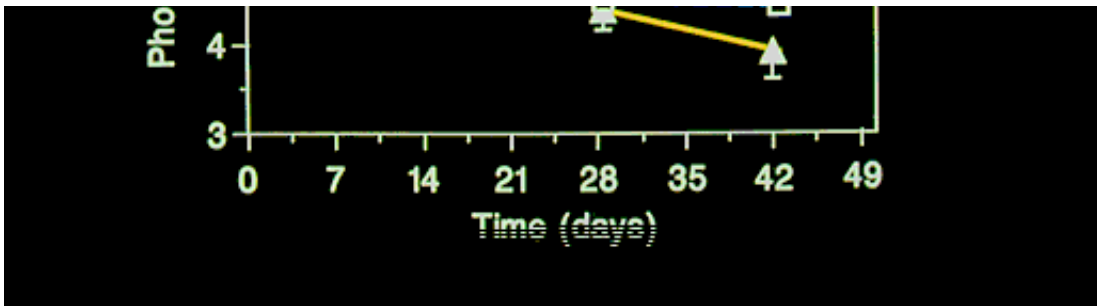
*Source: Naveh-Many T et al, J Clin Invest 1995 Oct;96(4):1786-93.*

**PCNA (Proliferative Cell Nuclear Antigen) is increased in uremic rats on a normal or high phosphorus diet, but prevented by a low phosphorus diet**

By a totally different methodology, Dr. Naveh-Many, working with Dr. Silver in Jerusalem, they measured PCNA. PCNA means proliferative cell nuclear antigen. It's a marker of cell mitosis. The more PCNA you have means that the cells are dividing more and more and more. If you look at normal parathyroid tissue, it's very quiescent, you don't see any division, and the number of mitosis are extremely low. If you make the animals uremic, the number of mitosis increases. If you make them uremic and feed a high phosphorus diet, the number of mitosis is even greater.

The point I want to make is not that one, but this one here. That uremic animals, who were fed a low phosphorus diet, the number of mitosis is the same as normal animals. Then, by two different types of methodologies, them and us, we clearly show that if we can restrict phosphorus early, we can prevent the development of chief cell hyperplasia.





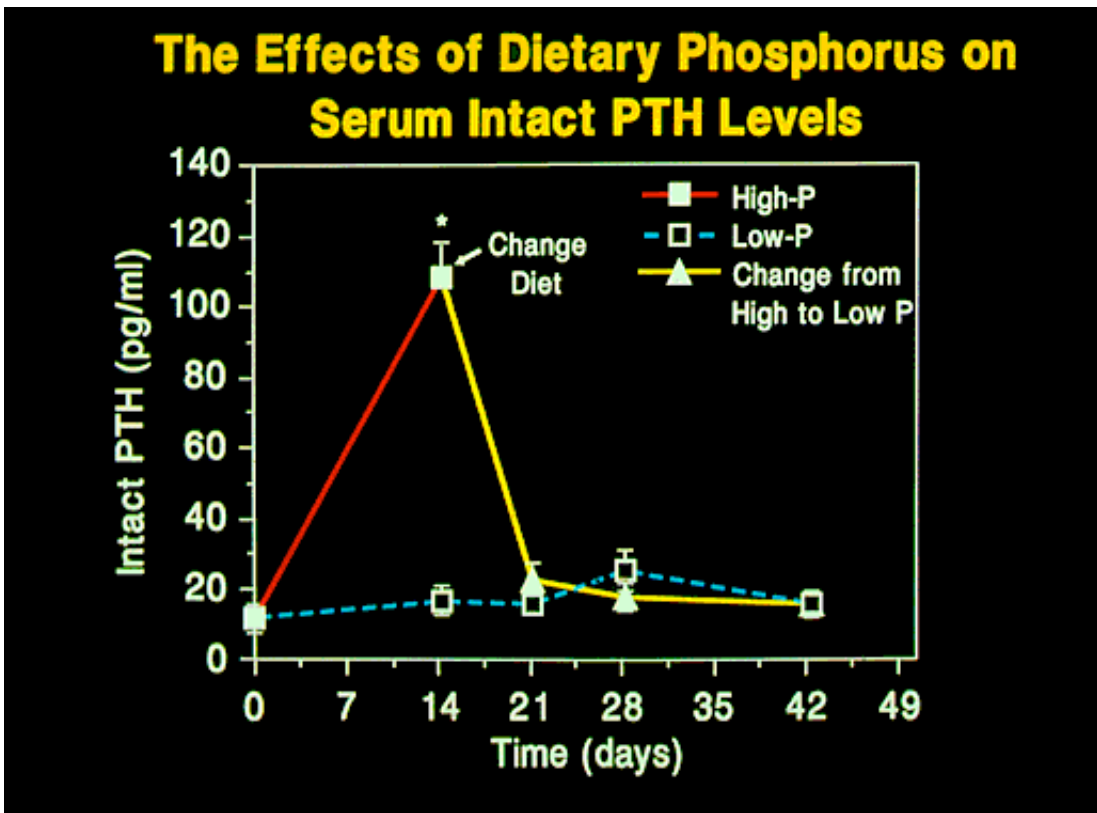
Source: Slatopolsky E et al, Am J Med Sci 1999 Jun;317(6):370-6.

### High-to-low phosphorus diet in uremic rats: effect on serum phosphate levels

The question that we ask ourselves is, if we have hyperphosphatemia, because we've fed the high phosphorus diet, what happens if now we change the diet, and we feed a low phosphorus diet. What changes will happen to the parathyroid glands?

Here you can see the animals on the low phosphorus diet, always in blue, they will have a low phosphorus; and a high phosphorus diet, of course, they develop hyperphosphatemia. And when we switch from red to yellow, it means that we go from a high to a low phosphorus diet. When we went here to a low phosphorus diet, of course, as suspected, the serum phosphorus will decrease. These are expected results according to the intake of phosphorus.

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Source: Slatopolsky E et al, Am J Med Sci 1999 Jun;317(6):370-6.

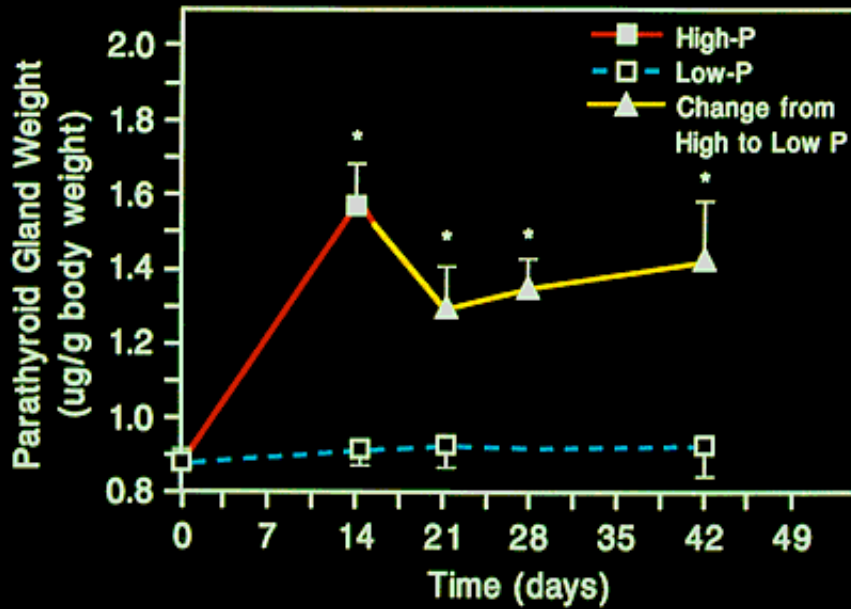
### High-to-low phosphorus diet in uremic rats: effect on serum PTH levels

What happened with PTH? In the animals on a low phosphorus diet, in blue, and we follow them now up to 90 days, they never developed secondary hyperparathyroidism. In red, as you cannot see it well, after two weeks on a high phosphorus diet, they have significant hyperparathyroidism. However, when we go from a high phosphorus to a low phosphorus diet, from red to yellow, PTH returns back to normal. Then we can reverse secondary hyperparathyroidism, at least in the rat.

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## The Effects of Dietary Phosphorus on Parathyroid Gland Weight

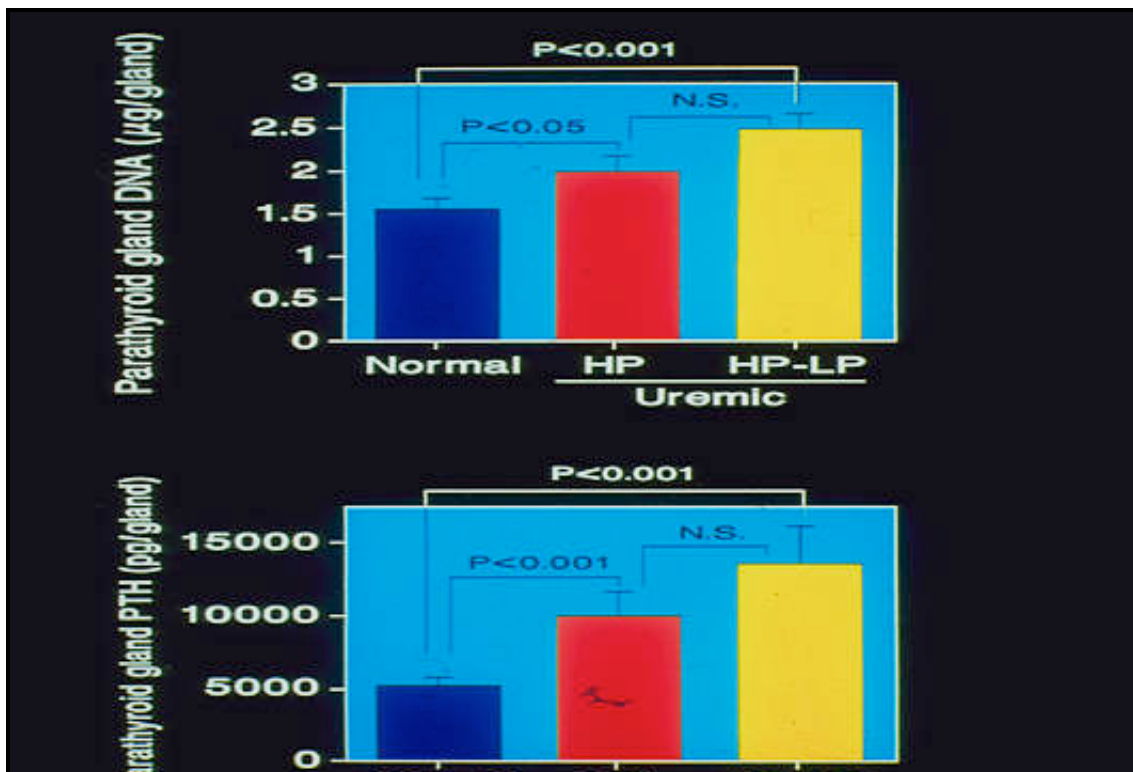


Source: Slatopolsky E et al, Am J Med Sci 1999 Jun;317(6):370-6.

### High-to-low phosphorus diet in uremic rats: effect on parathyroid gland size

However, next slide please, when we look at the size of the parathyroid glands, they never increase in the low phosphorus diet, and increase, and there is hyperplasia in a high phosphorus diet. When we switch them back to a low phosphorus diet, we do not reduce the size of the parathyroid glands. The glands remain enlarged in size. Although temporarily we may control PTH, the glands still are very large. And the question that we'd like to ask is, how can we have large glands and there is no PTH in the serum?

00:00



Normal HP HP-LP Uremic

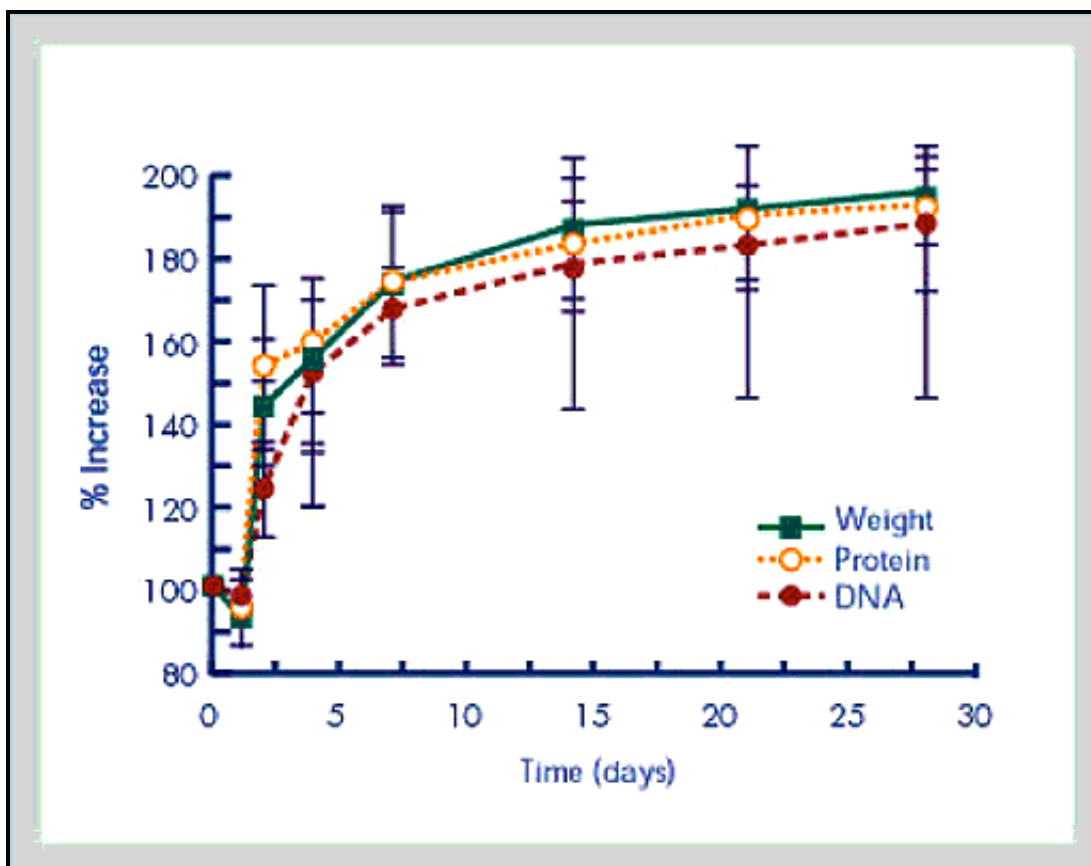
Source: Slatopolsky E et al, Am J Med Sci 1999 Jun;317(6):370-6.

### High-to-low phosphorus diet in uremic rats: effect on intracellular PTH levels

Then we measure intracellular PTH, next one please, and this is the amount of intracellular PTH in normal animals, 5000 picograms, there is 10,000 in the animals ingesting the high phosphorus diet, and when we go from the high phosphorus to a low phosphorus, when there is no PTH in serum, the gland is loaded with PTH. In other words, we cannot cure secondary hyperparathyroidism, we cannot cure hyperplasia of the parathyroid gland with a low phosphorus diet. We can suppress the secondary hyperparathyroidism, like you cannot cure diabetes because you give insulin. You control the sugar. We can control the PTH.

Now, in uremic patients, it's a little different, because they have huge glands. They have monoclonal changes, microvascular changes, nodular changes, and lower densities of calcium and vitamin D receptors. By controlling phosphorus alone, although you improve the PTH, although you make things easy for the nephrologist to use a vitamin D analogue with just controlling phosphorus alone, you cannot reverse the PTH back to normal.

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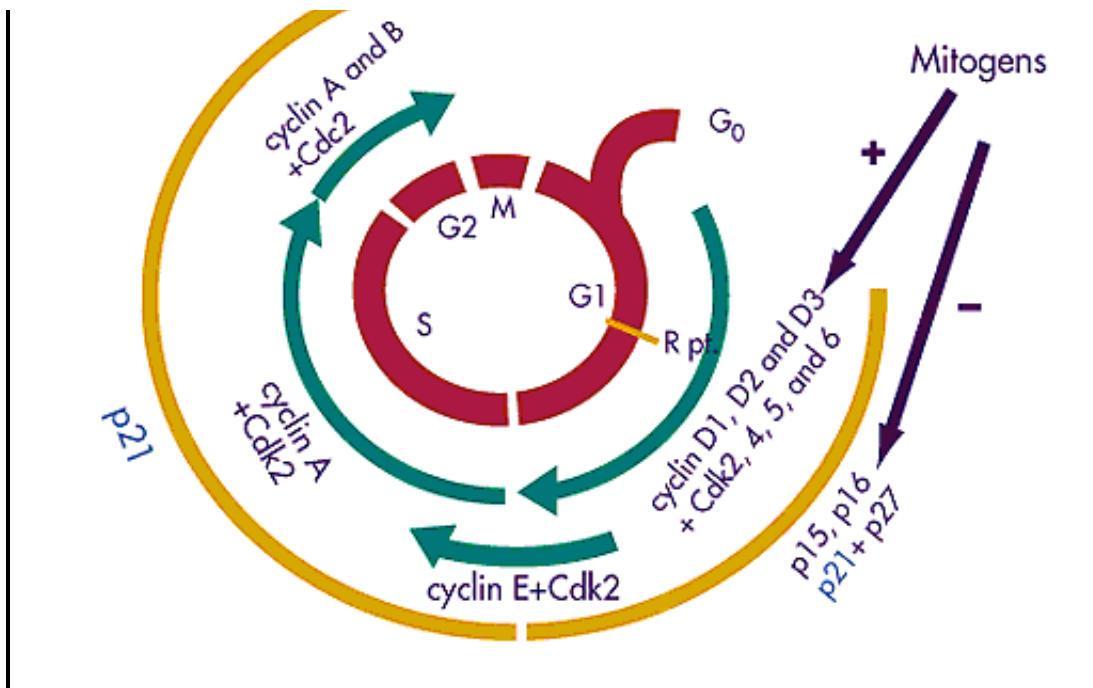
Source: Slatopolsky E et al, Am J Med Sci 1999 Jun;317(6):370-6.

### It only takes about 5 days to develop parathyroid hyperplasia in uremic rats

Now, how long does it take for the animal to develop hyperplasia? On the low phosphorus diet, they never develop hyperplasia. To our surprise, in another study, we killed the rats every two or three days, in four or five days we found hyperplasia. Then the action is here, in the first four or five days.

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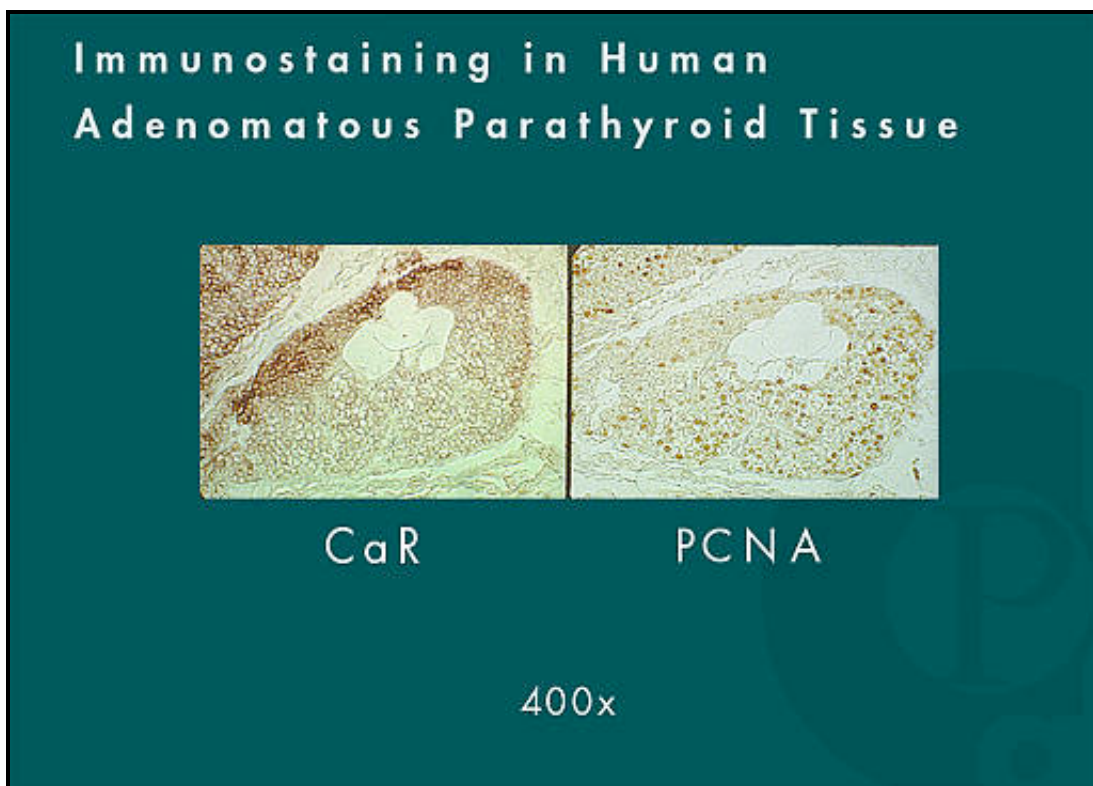


Source: Dusso AS et al, *J Am Soc Nephrol (abstract)* 10:617A, 1999.

### Low phosphorus may suppress cell cycle changes associated with mitosis

What happens is, the cell cycle is changed. And low phosphorus suppresses cell cycle, and high phosphorus induces the cell cycle. And Dr. Dusso will present in great detail that a suppressor of the cell cycle, p21, is induced by the low phosphorus diet. And TGF-alpha, that it increases proliferation is enhanced by the high phosphorus diet. For those of you who would like to learn at the molecular level, her paper will be presented tomorrow at 4:00 p.m.

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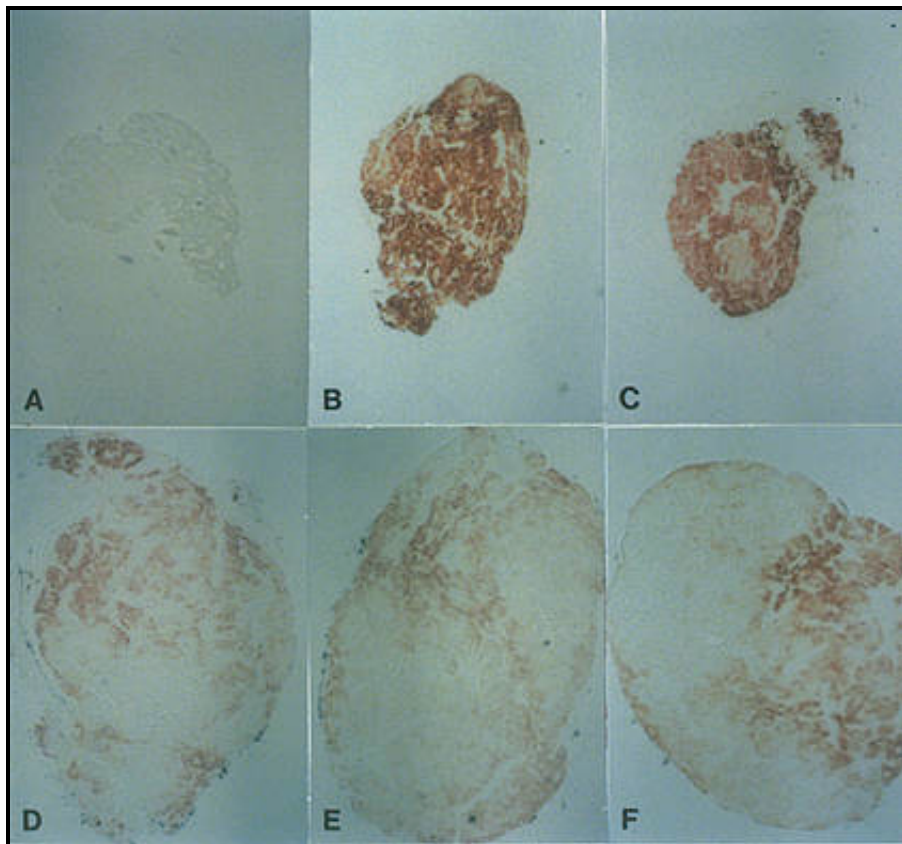


Source: Brown AJ et al, *Kidney Int* 1999 Apr;55(4):1284-92.

Calcium sensors in the parathyroid gland: detection by immunostaining

With all the calcium sensor, this is a normal parathyroid gland, and here we have a stain, the parathyroid gland with a specific antibody, for the calcium sensor. And the study has been done by Cindy Ritter and Alex Brown in our laboratory.

00:00



*Source: Brown AJ et al, Kidney Int 1999 Apr;55(4):1284-92.*

**Calcium receptor density is reduced in enlarged uremic parathyroid glands**

As you can see, here we have a pre-immune serum, that's why you don't see anything. Here we have an antibody of the normal parathyroid glands, and when we look at parathyroid glands from uremic animals, as you can see they are much larger, and the color is very light. In other words, we have lost substantial amount of the calcium sensor. Then we decided to study the effect of phosphorus on the calcium sensor.

00:00

## Diets for Uremic Rat Study

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**Normal Rats: Normal P (0.5% P, 0.9% Ca)**

**Uremic Rats: Low P (0.2% P, 0.5% Ca)**

**Normal P (0.5% P, 0.9% Ca)**

**High P (1.2% P, 0.4% Ca)**

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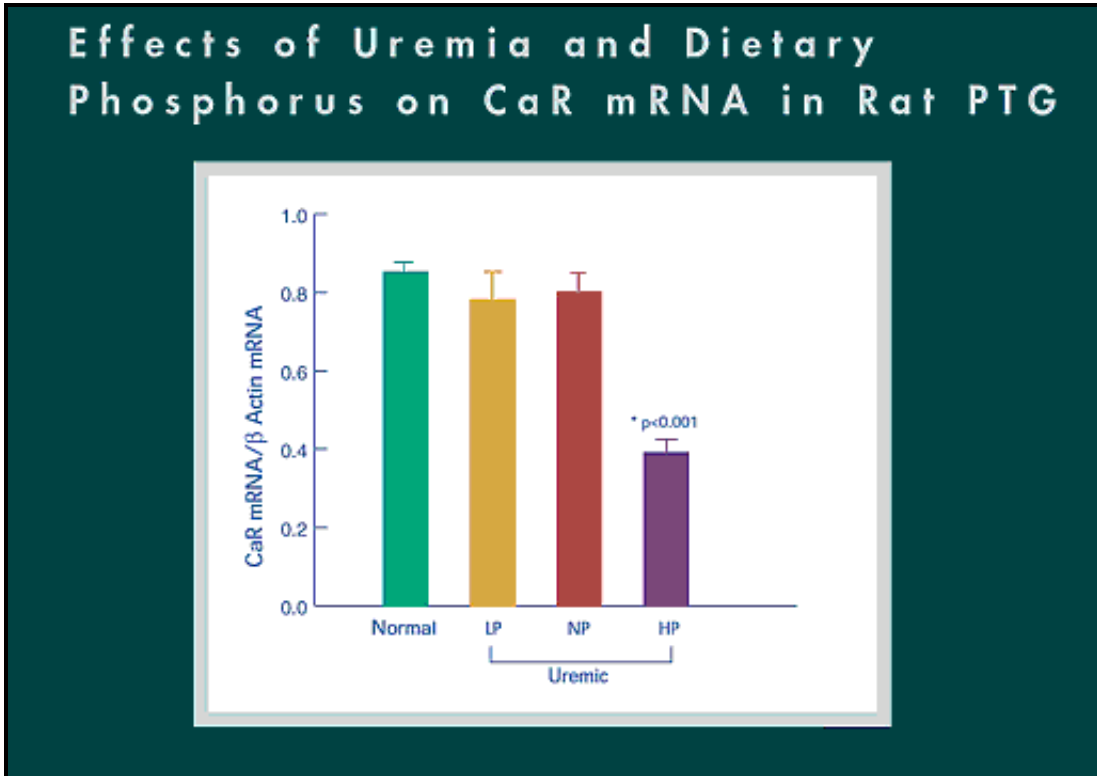
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Source: Brown AJ et al, *Kidney Int* 1999 Apr;55(4):1284-92.

### Effects of low / normal / high phosphorus diet on Ca sensing receptor mRNA and density by immunostaining

We used normal rats, uremic rats, and a low, normal, and high phosphorus diet.

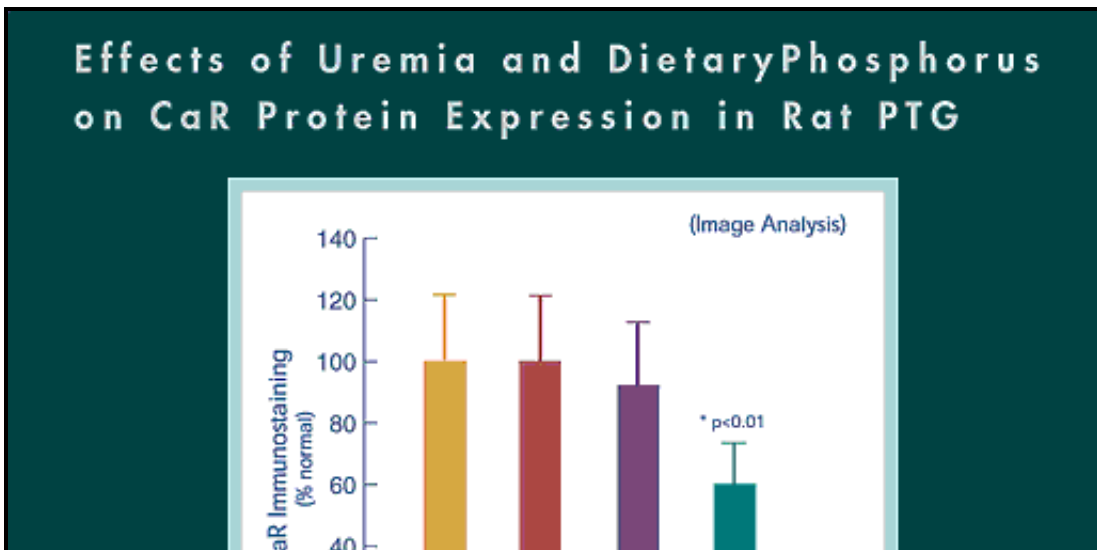
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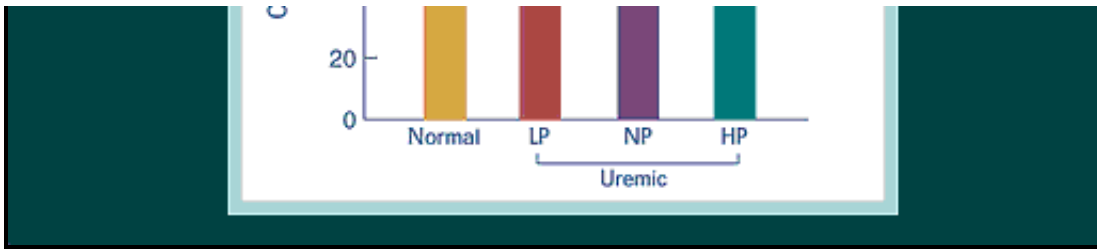


Source: Brown AJ et al, *Kidney Int* 1999 Apr;55(4):1284-92.

When we measure mRNA, for the calcium sensor was normal in the normal animals, of course, and in uremic animals on a normal or low phosphorus diet. But it was very low in animals on the high phosphorus diet.

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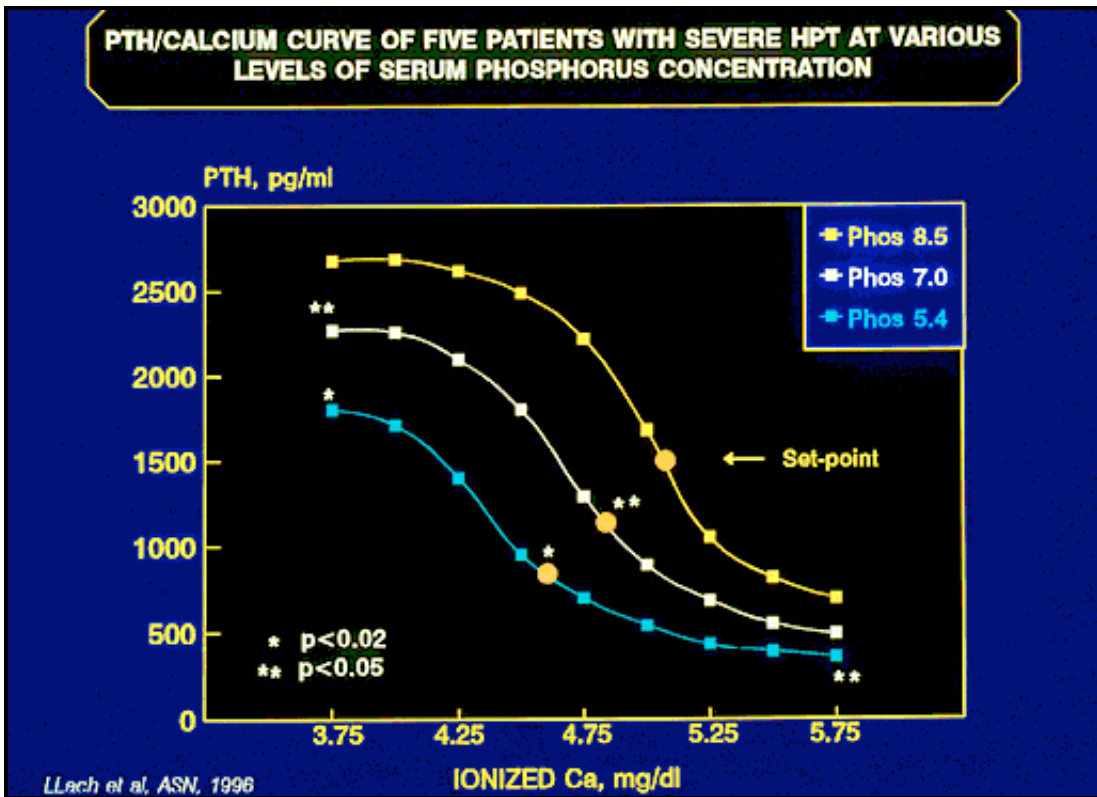


Source: Brown AJ et al, *Kidney Int* 1999 Apr;55(4):1284-92.

### High phosphorus diet reduces Ca sensing receptor density by immunostaining

When we did histochemical staining, these are normal animals, as you see, we can lose the receptor in the uremic animals on a high phosphorus diet, but there is a significant improvement on a low phosphorus or normal phosphorus diet. And when we did the image analysis with a computer, we can see that the only one that has a very low number of receptors is the uremic animals on a low phosphorus diet. That's by reducing the amount of phosphorus, we may bring the receptor back to normal.

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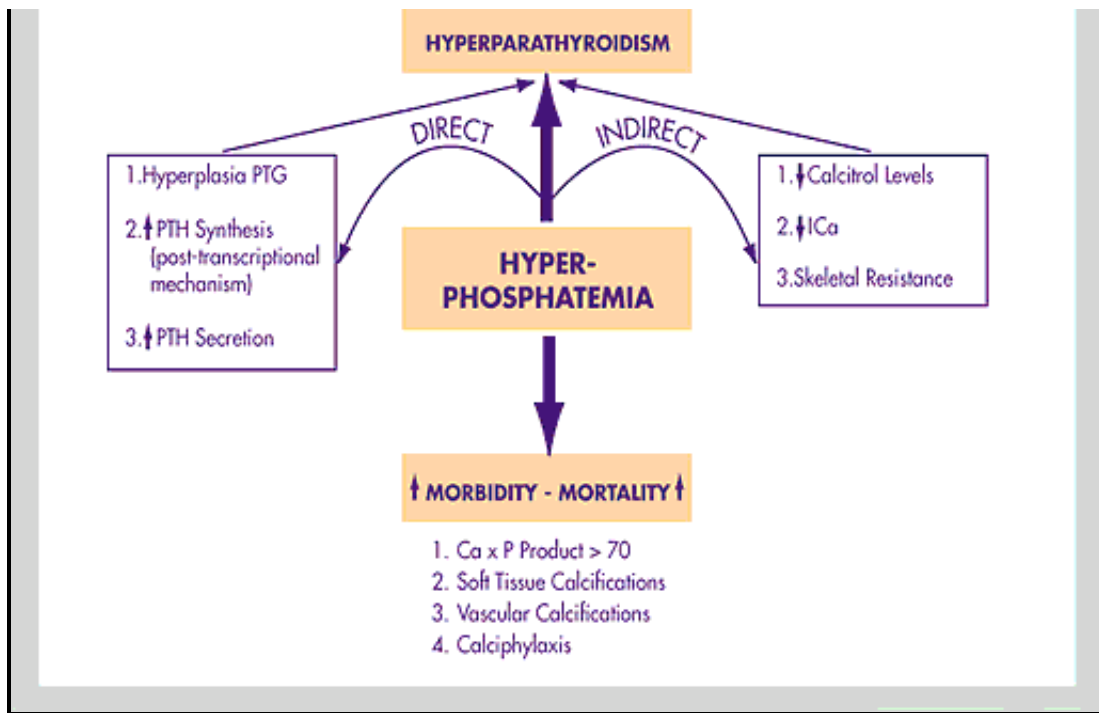
Llach et al, ASN, 1996

Source: Llach F and Yudd M. *Nephrol Dial Transplant* 1998;13 Suppl 3:57-61.

### Effect of phosphorus on Ca/PTH setpoint

And, I want to right past the next two slides. I want to show you this picture from Dr. Liage, because sometimes it's difficult to correlate the studies in vitro and in vivo, this is where a study is done in vivo in patients, which, he measures set point in a group of patients at different concentrations of phosphorus. And when he reduced the amount of phosphorus in these patients by being very aggressive and got the phosphorus down from 8.5 to 7.0 and 5.5, not only is there a mild decrease in the levels of PTH, as you can see, the PTH is still very, very high. But, there is an improvement in the setpoint. Perhaps, and there is no way I can prove that, that this could be correlated with the fact that we may increase the number of receptors, calcium receptors, at least we see this in our rats.

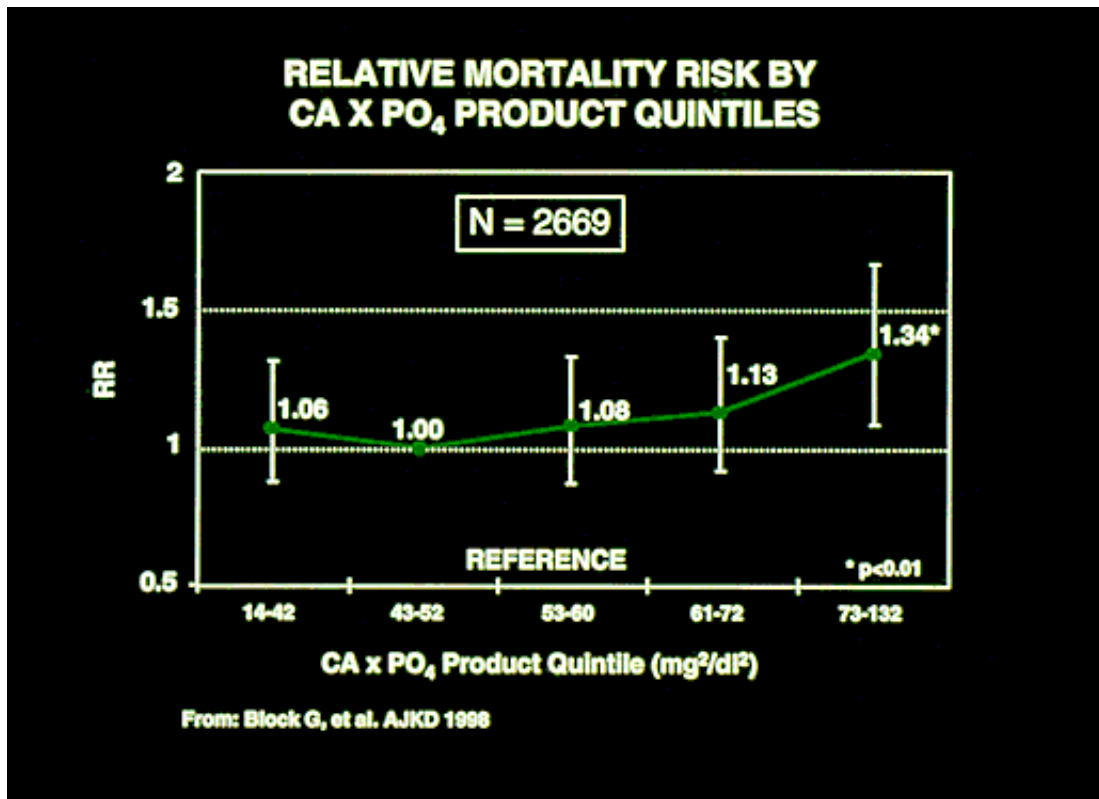
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**Direct vs. indirect mechanisms whereby hyperphosphatemia induces secondary hyperparathyroidism**

Now I want to summarize here by saying hyperphosphatemia induces hyperparathyroidism by two independent mechanisms. One indirect, known by everybody for the past 20, 25 years, by decreasing the levels of calcitriol, by decreasing the levels of calcium, and by inducing skeletal resistance. And directly, by producing chief cell hyperplasia, by increasing the synthesis of PTH, and therefore by increasing the secretion of PTH.

00:00



Source: Block GA et al, Am J Kidney Dis 1998 Apr;31(4):607-17.

Outcomes data: increased mortality associated with elevated Ca x P product

But now we are also paying attention in another problem, mortality and morbidity. Dr. Block has shown that with a calcium phosphate product greater than 70, mortality increases. And as you well know, the high mortality in dialysis patients is due to cardiovascular effect, these patients have accelerated atherosclerosis. And if they have a high calcium phosphate product, they can calcify the atherosclerotic plaques. Then we try to control this serum phosphorus and calcium phosphate product as you will see in a minute.

I'd like to emphasize one problem, which is a serious one, of calciphylaxis. Calciphylaxis is a very complicated, serious complications with high mortality of 60 to 90 percent. Not always is it due to high calcium phosphate PTH product. Not always. But in a large number of patients it is. And I want to emphasize that we want to avoid, as much as we can, this problem that can be induced by high calcium phosphate product.

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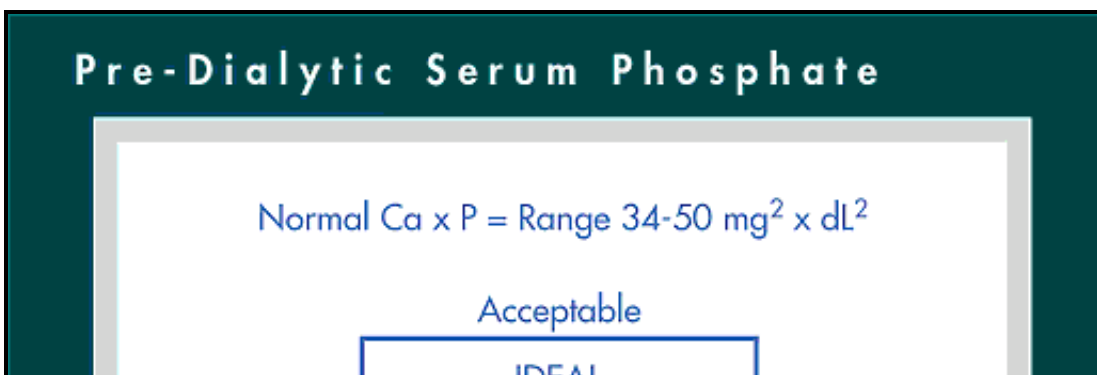


### Calciphylaxis

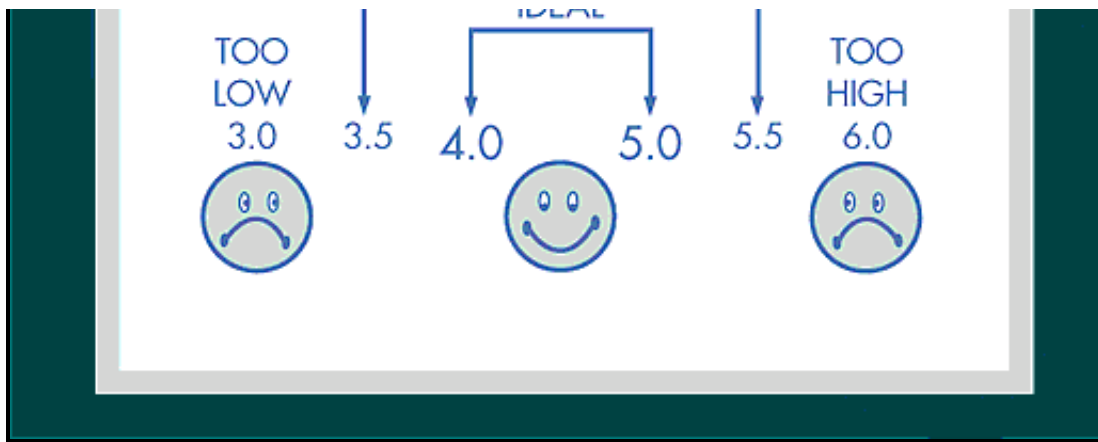
This mortality will be discussed in great detail by Dr. Chertow, so I'm not going to talk about this patient, but this is the type of problem that was I was referring to you, the calcification of the vessels. I apologize for this slide for your dinner, I hope you can take your dinner too. Sloughing of the skin.

In a recent paper published by Dr. Amann and Dr. Eberhard Ritz, they found that after a few months that a patient is in dialysis, gets severe calcification of the aortic valve. Then it is critical that we maintain a lower calcium phosphate product.

00:00







### Concluding remarks: What is the desirable Ca x P product?

And I'd like to finish my presentation with the last slide to emphasize that if you multiply the upper limit of calcium by the upper limit of phosphorus, it's not 70, it's 50. And we have accepted for a long time that a good calcium phosphate product was 70 to 75. I think that we have to change our approach.

In the past we used aluminum and we were wrong. We induced osteomalacia. In the past we used calcium carbonate and we thought that we were doing a good job, but we got a very high calcium phosphate product.

Today we can drop the serum phosphorus without producing hypercalcemia. And that will help us because if we keep the calcium phosphate product at a lower concentration, we will be able to use new vitamin D analogues, that produce less hypercalcemia and less hyperphosphatemia. That the combination of the two new drugs, the new phosphate binders, and the new vitamin D analogues, is going to help physicians to control secondary hyperparathyroidism. I'm going to finish here, and thank you very much.

### References

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