INTESTINAL CALCIUM ABSORPTION, 25-OHD₃ AND PARATHYROID HORMONE AT DIFFERENT STAGES OF RENAL INSUFFICIENCY

R-D Hesch, R Hehrmann, H Jüppner, R Wilke, K Lustenberger
Medizinische Hochschule Hannover, Hannover, Germany

It is difficult to give a complete review of disturbed mineral handling by the diseased gut in renal insufficiency. Disturbed calcium metabolism is the most prevalent disorder, with the most important clinical consequences.

Calcium Transport

a) Calcium transport and hence intestinal calcium absorption in the gut is under the influence of many, but only partly understood, control mechanisms (Table I). Under physiological conditions the entire small intestine may transport calcium against a concentration gradient, but the rate of transport in the distal intestine is lower than that in the upper duodenum (Krawitt & Schedl, 1968). Low dietary calcium increases calcium absorption by an adaptive mechanism in the entire small intestine (Kimberg et al, 1961; Ireland & Fordtran, 1973). Calcium absorption is intimately linked to the ratio of calcium to phosphate in the diet, especially at low dietary concentrations of calcium (Clark, 1969; Walling & Rothman, 1969). Adaptive mechanisms of calcium transport across the intestinal wall involve a two step procedure, both exclusively under the control of para-
thyroid hormone and D-hormone metabolites. First, active uptake of calcium by the microvillar membranes depends on the stimulation of local alkaline phosphatase and energy-depending adenosine triphosphatase (Melancon, DeLuca, 1970), and also a passive diffusion of calcium at this site of transport has been suggested. Transepithelial transport is mediated by a calcium-binding-protein synthesised via direct RNA effects of D-hormone metabolites. Serosal transport is thought to be a passive diffusion process (Review: Avioli, 1972) but this step probably may not be operative in vivo where there is a transport through the local blood vessel membranes.

b) Toxic products. The situation of disturbed calcium absorption in renal insufficiency is quite complex because we must assume several toxic products found in uraemia which alter calcium absorption directly at the site of active transport (Table II). These toxic effects act upon both mucosal uptake at the

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<tr>
<th>TABLE II. Toxic Effects on Intestinal Calcium Absorption in Renal Insufficiency</th>
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<tr>
<td>Depression of alkaline phosphatase from the brush-border-fraction</td>
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<tr>
<td>Depression of calcium-activated ATP-ase from the brush-border-fraction (zinc, copper, magnesium, aluminium?)</td>
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<tr>
<td>Toxic effects of methylguanidine (and urea?)</td>
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<tr>
<td>Ammonia (hypoacidity through neutralisation of gastric acid)</td>
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<tr>
<td>Sulphate (?)</td>
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<tr>
<td>Acidosis</td>
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surface and on the rate-limiting process of transfer towards the cell and the serosal surface (Gitelman, 1970). The biochemistry of these energy dependent steps is increasingly disturbed when renal insufficiency progresses but an exact elucidation of these effects is not yet possible because the importance of potential toxic agents has yet to be established. The alteration of calcium transport would seem to be specific, since the transport of hexoses and L-leucine is not affected. Calcium-binding protein and the vitamin-D dependent steps in calcium absorption do not appear to be involved (Baerg et al, 1970), the same being true for barium and strontium absorption. It should be mentioned that such toxic effects on intestinal calcium absorption seem to develop rapidly in acute renal failure since we could already observe the abolition of $^{47}$Ca-absorption two weeks after the onset of acute uraemia in one subject (Hesch et al, 1971). It is important to mention that although increased levels of methylguanidine, urea, creatinine can successfully be treated by dialysis and dietary manipulations (Giovanetti et al, 1970) calcium absorption remains disturbed (Hesch et al, 1972). The direct toxic effects, therefore, of the, as yet, poorly defined uraemic toxins (Black, 1970) are still speculative, since there are no data on toxic intra-
cellular effects, which certainly reflect biochemical alterations far better than the study of the extracellular milieu.

Dietary Factors

Apart from these considerations therapeutic manipulations alter calcium absorption leading to long term negative calcium balance.

a) Most important in this regard is aluminium hydroxide, which binds phosphate and hence calcium.

Inorganic phosphate arises from intestinal absorption, phosphoprotein and phospholipid metabolism, from increased bone turnover and is directly related to tubular function in the diseased kidney. High phosphate concentrations influence calcium absorption (Clark, 1969; Walling & Rothmann, 1969). Dietary manipulations and dialysis have only moderate effects on phosphate concentration, whereas effective treatment with aluminium hydroxide and suppression of hyperparathyroidism are most important. The long term side effects of aluminium are not yet fully elucidated but they may have clinical significance. It is still unclear whether high phosphate is itself responsible for the onset of hyperparathyroidism or not. From our early results (Hesch et al, 1972) and more recent experiences, disturbed intestinal calcium absorption with consecutive hyperparathyroidism is already present in early renal failure when serum phosphate is not significantly elevated. We regard, therefore, high phosphate, not as a primary event in renal calcipathy, but as a severe complication in the development of disturbed calcium metabolism with progressing renal failure. A critical concentration of phosphate and calcium in combination with acidosis, critical local concentration of D-hormone metabolites and parathyroid hormone lead to the deleterious soft tissue and arterial calcification.

b) The main effects of protein-deficient diet (Giovanetti et al, 1970) are on urate, guanidinosuccinic acid, methylguanidine, creatinine, sulphate, phosphate and hydrogen ions. Phosphate, sulphate, methylguanidine and acidosis potentially influence calcium absorption (Lestradet et al, 1962). The benefit of dietary effects on intestinal absorption is obviously much less than the side effects of vitamin D depletion (Offermann, 1975). Dietary effects of vitamin D depletion became mostly expressed in advanced renal failure and represent therefore a typical complication of this otherwise effective therapy.

Magnesium

The ratio of calcium to magnesium in the diet can vary considerably. High concentrations of magnesium in renal insufficiency can be dangerous and arise from antacids containing magnesium (~up to 5 g/day). Renal failure per se is not accompanied by clinically significant disorders of magnesium metabolism. Very
little, however, is known about possible effects of magnesium on the bioavailability of PTH. It has been claimed that a dialysis concentration of 2.5 mEq/l of magnesium may protect against soft tissue calcification, possibly counteracting the local effects of calcium, D-hormones and PTH.

Metabolism of Antirachitic Hormones

Vitamin-D, 25-OHD$_3$ and the metabolism of antirachitic hormones are severely disturbed during the development of renal insufficiency. These alterations have been the subject of numerous investigations in the last few years and have been summarised recently at the workshop conference at Wiesbaden (Norman et al, 1975). Hydroxylation of cholecalciferol in the liver is not disturbed in renal insufficiency provided that the dietary supplement of cholecalciferol is well balanced. Protein restricted diet, however — a necessary treatment — reduces the essential intake of vitamin D and leads to what is called ‘low vitamin-status’ in patients with advanced renal failure (Offermann & Dittmar, 1975). Although 25-OHD$_3$ measurement reflects only to a certain extent the whole body pool (vitamin-D-status), it provides the best estimation currently available (Stanbury et al, 1975). 25-OHD$_3$ is neither correlated with the expression of uraemic bone disease nor with serum calcium levels but, as would have been expected, there is a negative correlation with immunochemically determined PTH in advanced renal failure. Treatment with high doses of vitamin D$_3$ increases 25-OHD$_3$ levels and is accompanied by a significant decrease of serum parathyroid hormone and increase of serum calcium. This accords with our early observations in patients undergoing dialysis where doses from 20,000 IU/day up to 100,000 IU/day of vitamin D$_3$ could restore intestinal $^{47}$Ca-absorption (Hesch et al, 1972). One must, therefore, assume a direct effect of 25-OHD$_3$ on PTH metabolism and this has recently been confirmed by Verberckmoes and Bouillon (1975). A possible direct effect of 25-OHD$_3$ may however also be explained by an increase of 1,25-(OH)$_2$D$_3$ by the remaining activity of 1-$\alpha$-hydroxylase in the diseased kidney. 1,25-(OH)$_2$D$_3$ is now generally considered as the most biologically active D-hormone.

1,25-(OH)$_2$D$_3$ is a tissue hormone from kidney produced from 25-OHD$_3$ by 1-$\alpha$-hydroxylase which is a mitochondrial enzyme. The formation of 1,25-(OH)$_2$D$_3$ depends on several important factors recently reviewed by Henry and Norman (1975). Most of these effects are summarised in Figure 8. Although not finally proven it appears realistic from clinical observations to suggest that diminished activity of the enzyme is different in glomerulonephritis and pyelonephritis. The expression of reduced activity depends, however, upon several other factors. Dietary calcium is a rate-limiting factor for enzyme activity and local phosphate concentration is important. The intimate interrelationship of PTH and acid-balance affects the complex situation of production of 1,25-(OH)$_2$D$_3$. Haussler (1975) was able to demonstrate directly that 1,25-(OH)$_2$D$_3$
is low in renal disease and undetectable in anephric subjects. The effects on bone, kidney and intestinal calcium-absorption in renal disease vary considerably. It is uncertain whether kidney resistance to PTH (von Lilienfeldt et al, 1974) is explained thereby. Intestinal calcium absorption may entirely depend on 1,25-(OH)₂D₃ concentration. Deficiency of 1,25-(OH)₂D₃ is only partly responsible for skeletal resistance of uremic bone to PTH (Massey et al, 1975). Probably vitamin D₃ and 25-(OH)D₃ have different effects on osteoblastic activity than does 1,25-(OH)₂D₃ and similar derivatives (Bordier et al, 1975). The conflicting histological features may be explained by the fact that osteoblastic expression and hence osteomalacia is mainly under the control of 25-OHD₃, whereas hyperparathyroidism depends more on the mediation of 1,25-(OH)₂D₃ and only moderately if at all on the 25-OHD₃ concentration at the receptor site, as recently demonstrated by our group (Hesch & Jüppner, 1976). Even with severe hyperparathyroidism, osteomalacia may be prevalent as is well known from our own clinical observations and from the literature (Arnaud et al, 1975). These considerations may have important therapeutic implications.

Secretion and Metabolism of Parathyroid Hormone

Secondary hyperparathyroidism is an obligatory complication of chronic renal disease. Heterogeneity of circulating PTH levels (Hesch et al, 1975, 1976) makes the situation more difficult. Apart from glandular heterogeneity, different receptor handling and cleavage are the unknown factors when immunochemically determined PTH levels are correlated with clinical, osseous and laboratory changes. Although determination of C-terminal fragments is clinically and bio-

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chemically the most reasonable measurement of PTH, due to the amplification mechanism of slow peripheral metabolism (Lustenberger et al, 1976), it is only suitable for the assessment of hyperparathyroidism. The importance of determining biologically active PTH (Hesch & Jüppner, 1976) for elucidation of direct interrelationship between calcium, phosphorus, metabolism of D-hormones and bone metabolism is as yet uncertain.

Verberckmoes and Bouillon (1975) have, however, already shown that N-terminal and C-terminal PTH behave differently with vitamin D therapy. We now have direct evidence (Lustenberger et al, 1976) that endogeneous PTH levels considerably influence PTH metabolism due to saturation of the hormone receptors and/or the degradation enzyme, a situation similar to that of glucagon (DeRubertis & Craven, 1976) and TSH (DeRubertis et al, 1976). This may have important effects on the metabolic expression of biologically active PTH which have yet to be considered in renal failure (Arnaud et al, 1975).

We would now discuss some practical aspects derived from recent results of our group. Figure 9 demonstrates $^{47}$Ca-intestinal-absorption, which decreases with the advance of renal failure. We could demonstrate that treatment with vitamin D$_3$ but also with 25-OHD$_3$ in doses of 18–36 μg/day can improve disturbed calcium absorption (Figure 10). The efficacy of the treatment is, however, limited in patients undergoing dialysis, because of secondary hyperparathyroidism, so that vitamin D treatment is only of minimal value in this group of patients. This accords with PTH values which are only occasionally fully suppressed under vitamin therapy, controlled by 25-OHD$_3$ levels (Offermann & Dittmar, 1975).
We have determined intact (1–84) PTH (Hesch et al, 1975), C-terminal PTH, 25-OHD₃, serum calcium, serum alkaline phosphatase at different stages of the development of renal failure and summarised these data in Table III. From the data we can conclude that alkaline phosphatase tends to be elevated when 25-OHD₃ is decreased and calcium also tends to be lowered when 25-OHD₃ is decreased in advanced renal failure. This is most probably due to dietary restrictions, a reduction of the vitamin D-status and consequently decreased intestinal calcium absorption.

Calcium is normal in patients with GFR 60–20 ml/min and in patients undergoing haemodialysis with normal 25-OHD₃. In both groups, however, normal

<table>
<thead>
<tr>
<th>Clinical state</th>
<th>C-terminal PTH (μl/eq)</th>
<th>1-84 (intact) PTH (μl/eq)</th>
<th>Calcium mEq/L</th>
<th>alkaline phosphatase (50-100)</th>
<th>25-OHD₃ ng/ml</th>
</tr>
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<tbody>
<tr>
<td>Normal range</td>
<td>Zero - 2.5</td>
<td>Zero 1.0 (?)</td>
<td>2.12-2.63</td>
<td>76-190</td>
<td>40-60</td>
</tr>
<tr>
<td>GFR 60-20 ml/min n=11</td>
<td>9.8±8.5 (all &gt; 2.5)</td>
<td>5.8±6.0 (all below 1.0)</td>
<td>2.49±0.12 (all normal)</td>
<td>156.9±63</td>
<td>32.8±21</td>
</tr>
<tr>
<td>GFR &lt; 10 ml/min n=23</td>
<td>11.9±7.8</td>
<td>8.5±7.3</td>
<td>2.12±0.3</td>
<td>201±109</td>
<td></td>
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<tr>
<td>C-ter. PTH &lt; 10</td>
<td>5.4±0.8</td>
<td>4.6±5.4</td>
<td>2.36±0.1</td>
<td>25±44</td>
<td></td>
</tr>
<tr>
<td>C-ter. PTH &gt; 10</td>
<td>20.0±3.6</td>
<td>12.0±7.1</td>
<td>1.82±0.1</td>
<td>185±27</td>
<td></td>
</tr>
<tr>
<td>1 - 84 PTH &lt; 5</td>
<td>7.3±4.9</td>
<td>2.0±1.3</td>
<td>2.25±0.3</td>
<td>174±92</td>
<td></td>
</tr>
<tr>
<td>1 - 84 PTH &gt; 5</td>
<td>14.5±7.0</td>
<td>14.1±5.6</td>
<td>2.10±0.5</td>
<td>277±170</td>
<td></td>
</tr>
<tr>
<td>Hemodialysis n=13</td>
<td>12.7±7.1</td>
<td>-</td>
<td>2.30±0.15</td>
<td>152±82</td>
<td>39.5±17</td>
</tr>
</tbody>
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Figure 10. Effect of Vitamin D₃ therapy in advanced renal failure on ⁴⁷Ca-absorption, serum calcium and phosphate. Increasing doses lead to normalisation in an increasing percentage of patients.
calcium values are associated with high PTH levels. This means that calcium is normalised by secondary hyperparathyroidism in both groups, following reduced intestinal calcium absorption and insufficient suppression of PTH secretion by decreasing concentrations of 1,25-(OH)₂D₃. In advanced renal failure low C-terminal PTH and 1–84 PTH are correlated with high serum calcium values, representing a population with less severe hyperparathyroidism and more pronounced osteomalacia. In another group, high C-terminal PTH values and 1–84 PTH values are inversely correlated with calcium levels demonstrating a more severe hyperparathyroidism in this group and supporting the view that the concentration of 1,25-(OH)₂D₃ at a low vitamin D-status determines whether osteomalacia or hyperparathyroidism is prevalent.

Under the conditions of haemodialysis and non-restricted diet with a normal vitamin D-status, parathyroid values are not suppressed (only 4 from 30 subjects had values below 5 μU/equiv) and we postulate autonomous hyperparathyroidism which is not suppressed by appropriate bath calcium concentrations. If 6 months after initiation of haemodialysis there is still autonomous hyperparathyroidism parathyroidectomy should be performed.

Summary

From these results we can derive a scheme for the clinical transitions during the development of chronic renal failure from the mild to the advanced form.

Figure 11 demonstrates the course of ⁴⁷Ca-intestinal absorption, calcium,
phosphorus and alkaline phosphatase. It should be noted that during transition I–II, calcium increases again, which is due to activation of 1α-hydroxylase (Arnaud et al, 1975) whereas calcium absorption remains disturbed and uninfluenced during dialysis (Hesch et al, 1972). Phosphorus is still normal at clinical state I. During transition II–III secondary hyperparathyroidism progresses to autonomy with hypercalcaemia.

Figure 12 portrays the course of 25-OHD₃, 1,25-(OH)₂D₃ and C-terminal PTH. The slight increase of 1,25-(OH)₂D₃ parallels that of calcium during transition I–II. 25-OHD₃ decreases continuously, indicating the development of the ‘negative vitamin-D-status’. The interrelationship between 25-OHD₃ levels, those of 1,25-(OH)₂D₃ and PTH together with calcium (intestinal absorption) and phosphate represent the actual dynamic background of renal osteopathy and renal calcipathy. During transition II–III autonomous hyperparathyroidism develops. Due to the slow kinetics of C-terminal PTH, however, suppression tests using determination of C-terminal PTH may give misleading results and indicate false non-suppressibility and hence autonomy. This question can actually only be answered by determination of 1–84 (intact) PTH or N-terminal fragments

![Diagram](image)

Figure 12. Serum concentration of 1,25-(OH)₂D₃, 25-OHD₃ and C-terminal PTH during the development of chronic renal failure

in sequence specific assays (Hesch et al, 1975) or by monitoring C-terminal PTH levels about 6 months after initiation of dialysis with high bath calcium concentration. If PTH remains high this will indicate autonomy, provided our current knowledge on peripheral metabolism of PTH peptides is correct. Figure 13 suggests prophylactic medical therapy of renal calcipathy on the base of our current
Figure 13. Recommendation for prophylactic therapy of renal calciopathy additionally to the established therapy with aluminium hydroxide. In mild and pre-dialysis renal failure oral calcium and Vitamin D₃ or 25-OHD₃ is recommended; in chronic haemodialysis high bath calcium concentrations (>3.5 mMol/litre) should be used. C-terminal PTH should be normal or only slightly elevated during all stages of renal failure.

knowledge. It implies early therapy in mild renal failure. This therapy can now be carefully monitored by controlling 25-OHD₃ plasma levels in conjunction with C-terminal PTH determination, measurements of plasma calcium, phosphorus, and alkaline and acid phosphatase. Measurements of intestinal calcium absorption and bone histomorphometry complete this scheme. Sufficient suppression of PTH with normal values for calcium, phosphorus and alkaline phosphatase will protect the patient against renal calciopathy during transition I–III. At stage III, high bath calcium concentration during dialysis continues to ensure equilibrated calcium balance, protecting against renal calciopathy. We would prefer therapy with vitamin D₃ itself or 25-OHD₃ on the base of the different particulate effects upon PTH, bone and intestinal absorption. Moreover determination of 25-OHD₃ helps to control the therapeutic effects safely. 1-α-hydroxylated derivatives are possibly dangerous due to uncontrolled 25-hydroxylation. We hope that renal calciopathy will soon be a disease of the past.

References

Avioli, LV (1972) Archives of Internal Medicine, 129, 345
Black, D (1970) Archives of Internal Medicine, 126, 906
Bordier, Ph J, Marie, P, Arnaud, CD, Gueris, J, Ferriere, Ch and Norman, AW — as first reference. Page 133
DeRubertis, FR, Chayoth, R, Zor, U and Field, JB (1975) Endocrinology, 96, 1579
Flueck, JA — as first reference. Page 397
Giovanetti, S, Balestri, PL, Biagini, M, Menichini, G and Rindi, P (1970) Archives of Internal Medicine, 126, 900
Gitelman, HJ (1970) Archives of Internal Medicine, 126, 793
Hausler, M — as first reference. Page 25
Henry, HL and Norman, AW — as first reference. Page 1
Hesch, R-D, McIntosh, CHS and Woodhead, JS (1975) Hormone and Metabolic Research, 7, 347
Hesch, R-D, Gerlach, W, Henning, HV, Emrich, D, Scheler, F and Kattermann, R (1972) Deutsche medizinische Wochenschrift, 45, 1735
Hesch, R-D and Jüppner, H (1976) V. ICE, Hamburg (abstract)
Hesch, R-D and Jüppner, H (1976) Endocrinology (In press)
Ireland, PI and Fordtran (1973) Journal of Clinical Investigation, 52, 2672
Lestradet, H, Frederick, A and Rodriguez-Soriano, J (1962) Presse médicale, 20, 211
Melancon, MJ and DeLuca, HJ (1970) Biochemistry, 9, 1658
Offermann, O and Dittmar, F — as first reference. Page 295
Stanbury, SW, Mawer, EB, Lumb, GA, Hill, LF, de Silva, P and Taylor, CM — as first reference. Page 205
Taylor, CM — as first reference. Page 205

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Open Discussion

CHAIRMAN Thank you very much Dr Hesch for this comprehensive paper. We will now discuss both papers relating to intestinal absorption.

COBURN (Los Angeles) I think Dr Hesch is to be congratulated for attempting to review a very complex matter and I think when one does this there is a necessity at times to be dogmatic about areas in which there are a large number of blind men looking at the elephant from different angles. There are several points I would like to make, where I would perhaps think that there is room for disagreement. First of all, in just considering mechanisms of calcium transport, data both from Haussler and from DeLuca show little correlation between the membrane calcium-activated ATPase and vitamin D stimulated calcium transport. I think both of these groups, who were the first to show that it was increased, now feel that it does not seem to correlate with increased transport. Now, second, a number of laboratories, the group in Iowa as well as Norman, feel that there is strong evidence that the serosal extrusion of calcium is active. For example, using the inert antibiotic filipin, one can apply this to the mucosal surface and exactly imitate vitamin D, take it away and it goes away without any change in activity — not that I am saying that vitamin D transport is not active, but it is as if once you get it in the cell it then sets off active metabolic steps. Now regarding the PTH effects on calcium transport, although there may be an effect, I think most people with the newer knowledge about vitamin D and 1,25 have really not been able to show an independent effect of parathyroid hormone on calcium transport.

Finally, I think the role of vitamin D in phosphate absorption has probably been underestimated. Years ago, people studying transport eliminated calcium from the serosal surface and saw no effect on phosphate. However, I think everyone now knows that one needs calcium next to the cell for all biologic functions. One can clearly show tremendous augmentation of phosphate transport when calcium is removed from the mucosal surface. As a matter of fact the increase in phosphate transport may be as much as five times the increased stimulation of calcium transport in some in vitro models, so I think we may have to refocus on what is primary, and there is evidence in chronic renal failure that phosphate absorption actually is decreased and it is increased by substituting active vitamin D forms. Now as far as your comments about osteomalacia occurring from low plasma levels of 25-hydroxy-vitamin D; most of the levels you show are levels above those in which osteomalacia has been reported in other conditions; thus, in other words, osteomalacia has usually appeared when levels have been lower than this. I guess the final question one might raise is whether one has excluded the possibility that this binding method for vitamin D might detect other forms such as 24, 25-dihydroxy vitamin D. I do not want to talk for ever, but there are a number of calcium transport mechanisms and one could spend a week discussing ramifications, but I think there are a number of areas of your comments about which not everyone would entirely agree.

HESCH Well, I will try to answer you point by point. Some of your comments I do not have to add anything to. I think the mechanism of the transport, where I have shown that it is not correlated to ATPase, may be true, but I have taken from the literature and may have been wrong.

The next thing is the serosal transport. What I think is that this transport mechanism probably may not be operative in vivo, because what you talk about
are probably mostly in vitro experiments with the everted sac. In vivo you do not have serosal transport because you have, rather, a transport in the vessels.

Another thing to consider is the influence of PTH on intestinal transport. I agree with you completely that it has never been truly shown that PTH really has an effect on intestinal transport. There is one study of Wills where they infused parathyroid hormone and have shown that intestinal calcium absorption is increased. We have tried to study this with the everted sac experiment, and we have shown that PTH in vitro has no effect on calcium transport. I think that in the Wills' study he has just infused PTH and stimulated the hydroxylation of 1,25 and then got the increase of calcium absorption, and I think that may be the true explanation for this mechanism.

Now to what you have been saying on vitamin D and its influence on phosphate transport, I would not like to add anything.

Coming back to your last and most important point — the 25 assay. Well, as you probably know the 25 assay has several complications: one is that 25 levels vary with the season, they vary extremely from one population to another and especially in Germany we have great problems because we have a lot of people from foreign countries who have completely different vitamin D status from German people. This is one point. Another point is the problem of stability of the standard, and we know that there are polar metabolites which can be preformed during the preparation of the standard. When you argue that perhaps we may be measuring 1,25 and 24-25 we would really be happy if we could do so. We have checked this out. I got 1,25 and 24-25 from DeLuca and have checked the assay for cross activity and there was no cross activity so we can really exclude that. Apart from that we do the ordinary Belsey assay with the modifications of Offerman. I cannot tell you more than that. The assay is very accurate at the moment, so I do not know why we have these higher values.

DRUEKE (Paris) I wanted to pose several questions to Dr Hesch. First of all, did you give vitamin D to anephric patients, and did you see any effect on calcium absorption after massive vitamin D doses?

HESCH No, we have never given vitamin D to anephric patients because we were not allowed, ethically, by our surgeon to do it.

DRUEKE Second question. You reported you did not see any effect of massive vitamin D doses on serum phosphorus, so indirectly you concluded that there was no effect of massive vitamin D doses on intestinal phosphorus absorption. We have given high doses of 25 hydroxycholecalciferol to uraemic patients and we have seen in more than half of the patients a marked increase in serum phosphorus, so I wanted to know whether you think there is a difference in the action of vitamin D and of 25, the more active metabolite, on intestinal phosphorus absorption.

HESCH I do not think so. I think your approach is a different one from ours. Our patients were receiving aluminium hydroxide treatment and this was continued, so I think that we would not see this effect on the vitamin D treatment.

DRUEKE No, I do not think it is different, as our patients were receiving identical treatment with aluminium hydroxide.

HESCH Then I cannot offer you another explanation. I am sorry.
DRUEKE Another question. You just mentioned in the discussion that you have done PTH experiments in vitro to study the possible direct effect of PTH on calcium transport. Did you give the PTH to the animals in vivo or did you incubate the PTH in vitro?

HESCH You mean calcium transport?

DRUEKE On calcium transport.

HESCH No, I have not said that. What I was saying is that we have studied the effect of 25 and 1,25(OH)₂D₃ on the receptor handling of PTH by renal tubular membranes, not on the gut membranes, and there we have found that 1,25 vitamin D but not 25-hydroxycalciferol inhibits the action of PTH on the receptor so, with increasing doses of 25-hydroxy vitamin D, PTH is not bound to the receptors.

DRUEKE Another question. You said that there is a strong interdependence of phosphorus and calcium absorption or there are papers in the literature which say that phosphorus concentration in the intestine does not directly influence intestinal calcium absorption. What do you think about this problem?

HESCH The only thing I can tell you is what I have found in the literature, that the relationship between phosphorus and calcium is extremely important for the absorption of calcium and that with high phosphate you get low intestinal calcium absorption and with low phosphate you get high calcium absorption. We may perhaps discuss what you mean by calcium absorption: it is not total absorption, but fractional absorption and this is something different.

DRUEKE Well, it is an indirect expression of the total absorption; of course you should do balance studies to find out all of this. May I put another question? First a question to Dr Grimmel now. When you say that there are differences between different groups of workers in the appreciation of lactose malabsorption, did you consider the fact that there are different ethnic populations of lactose malabsorption and that, for instance, in black people in the United States, if I remember well, there is up to 80% of lactose malabsorption, so might the differences between groups of workers be due to different ethnic populations?

GRIMMEL Yes, indeed it is so, but the first investigation by Madzarovova-Nohejlova was done in a population that is not different from the European population, and there we see lactose malabsorption in 20% of all people as a maximum. In our patients we have not seen it at all.

DENNEBERG (Sweden) May I ask Dr Grimmel about his beautiful experimental model. The results confirmed ours in human beings. May I ask at what stage were the animals killed and how long were they uraemic?

GRIMMEL We have 5/6 nephrectomised and have killed the rats at 60 days after the operation; and we have seen urea levels between 50 and 125, the mean 60 mg/100 ml.

DENNEBERG If I am right, Ricken found some differences between our