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THE SERUM HORMONE LEVELS, PHOSPHATE COMPLEX CONCENTRATIONS AND ENZYME ACTIVITIES IN HAEMODIALYSED AND KIDNEY-TRANSPLANTED CHILDREN

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The serum hormone (T3, FT3, T4, FT4, TSH, hTG, a-hTG, GH, PTH, PRL, Cortisol) concentrations, the inorganic phosphate complexes (HPO^{2-}_4, $H_2PO^-_4$, $NaHPO^-_4$, $KHPO^-_4$, $CaHPO_4$, $MgHPO_4$) and the enzyme activities (Amylase, Lipase, AP, ACE, GOT, GPT, Ψ-ChE, CK, γ-GT, LDH) were investigated in 13 haemodialysed children, 7 kidney-transplanted children and in 15 healthy controls. This study confirmed that the kidney plays an important role in the metabolism of hormones. Prior to kidney transplantation 8 of the 11 tested hormone levels of haemodialysed children significantly differed from those of healthy controls, however, after kidney transplantation only two parameters did. The effect of dialysis is the least on the $CaHPO_4$ complex among the different inorganic phosphate complexes. This may play a role in vascular calcification in chronic renal failure patients. The amylase and lipase activity were elevated in haemodialysed group, while in kidney-transplanted children the angiotensin converting enzyme (ACE) and alkaline phosphatase (AP) differed from those of the control group.

INTRODUCTION

The endocrine function of the kidney may be regarded either as primary, because the kidney is an endocrine organ producing hormones, or as secondary, because the kidney is a site where hormones produced elsewhere are inactivated. In its primary endocrine function, the kidney produces renin, prostaglandins and erythropoietin. In the seco but no less important aspect of its endocrine function, the kidney is a site of degradation of insulin, glucagon, aldosterone etc. Furthermore,

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the kidney is the location of the important 1-hydroxylation of 25/OH/-D_3 that produces 1,25/OH/$_2$-D_3, the most active known form of vitamin-D /26/.

Patients with chronic renal failure treated by haemodialysis often have symptoms of endocrine dysfunction /2/. The diagnosis or exclusion of an endocrine disorder in such patients is complicated by the presence of abnormal serum hormone levels. The aim of this study was to establish the effect of chronic renal failure and kidney transplantation to serum hormone levels in children. Comparison of literature reference values are often complicated because of the number of methodical modifications, so we investigated healthy children as controls, respectively. We had the possibility to measure the following hormones:
Total Triiodothyronine (T3), Free Triiodothyronine (FT3), Total Thyroxine (T4), Free Thyroxine (FT4), Thyroid Stimulating Hormone (TSH), Human Thyroglobulin (hTG), Anti Thyroglobulin Antibodies (a-hTG), Growth Hormone (GH), Cortisol, Parathyroid Hormone (PTH) and Prolactin (PRL).

Phosphate retention is commonly associated with chronic renal failure and has been implicated in the pathogenesis of secondary hyperparathyroidism and renal osteodystrophy /19, 23/. In this investigation, we calculated the individual inorganic phosphate components (HPO^{2-}_4, $H_2PO^-_4$, $NaHPO^-_4$, $KHPO^-_4$, $CaHPO_4$ and $MgHPO_4$) in serum obtained from patients before and after haemodialysis.

There are few data on the evolution of serum enzymes activity in children on renal replacement therapy /18/. Enzyme values are providing information on organ function, beside endocrine parameters, therefore the routinely used enzyme activities were compared, namely:
Amylase, Lipase, Alkaline Phosphatase (AP), Angiotensin Converting Enzyme (ACE), Aspartate Transaminase (GOT, ASAT), Alanin Transaminase (GPT, ALAT), Ψ -Cholinesterase (Ψ -ChE), Creatine Kinase (CK), γ-Glutamil Transferase (γ-GT) and Lactate Dehydrogenase (LDH).

PATIENTS AND METHODS

Thirteen haemodialysed children (7 females, 6 males) were enrolled into the study. Their age ranged between 8 and 17 years (mean 12 years) and mean duration of haemodialysis was 2 years. Renal failure etiology was: 3 hydronephrosis, 4 hypoplastic kidneys, 4 chronic glomerulonephritis, 1 nephrocalcinosis and 1 nephronophtisis. They were scheduled for dialysis on every second-third day with GAMBRO AK-10 instrument for a duration of about 3-4 hours.

Seven kidney-transplanted children have been examined with good graft functions, their age was 13 $\pm$ 2 years, mean duration of transplantation 14 months. The source of the kidney was in five cases living donor (parents) in two cases cadaver.

Fifteen healthy, age and sex matched children served as control for laboratory parameters.

Serum concentrations of creatinine, urea, sodium, potassium, calcium, magnesium, inorganic phosphate were obtained by standard clinical chemistry methods with the RA-1000 Analyser (Technicon Instrument Corp.). Serum hormone concentrations were measured by following methods: T3, T4 by Izinta ^{125}I-RIA tests, FT3, FT4 by Amersham ^{125}I-RIA tests, TSH, GH, PRL, Cortisol by Serono ^{125}I-IRMA and ^{125}I-RIA tests, a-hTG by Biodata ^{125}I-RIA test, hTG by Sorn ^{125}I-IRMA test. The calculation of various inorganic phosphate constituents was described previously /21/. Serum enzyme activities were measured by following methods: ACE, AP, Ψ-ChE, CK, GOT, GPT, γ-GT, Lipase by kinetic photometric method with Boehringer kits, Amylase by Phadebas test (Pharmacia Comp). Student's t-test was used for the statistical analysis of the data.

RESULTS

Table I shows the result of serum hormone measurements. The mean serum T3, FT3, T4, FT4, hTG were considerably lower in the haemodialysed patients than in the control children. There was no significant difference between the TSH and a-hTG concentration. In kidney-transplanted children even the FT3 and FT4 levels were normal. Figure 1 shows the change of thyroid hormone levels in the case of a 12 year old kidney-transplanted girl. This well demonstrates the regulation of T4/FT4~TSH system.

The other data of Table I are showing a slight increase of cortisol concentration which may be explained with the "stress effect" of dialysis at haemodialysed patients. In our study we did not find any change in growth hormone levels. The PRL

TABLE I

Results of serum hormone concentrations (mean ± SD)

Serum parameters		Control children	Haemodialysed children	Significance vs.control	Transplanted children	Significance vs.control
T3	nmol/l	2.7±0.5	1.5±0.5	$p < 0.001$	2.1±0.5	$p < 0.05$
FT3	pmol/l	7.3±1.6	4.4±1.6	$p < 0.001$	7.0±1.3	NS
T4	nmol/l	136±26	106±28	$p < 0.01$	110±21	$p < 0.05$
FT4	pmol/l	16.4±2.6	13.5±4.1	$p < 0.05$	17.8±3.5	NS
TSH	mU/l	2.8±1.6	2.5±1.2	NS	1.7±0.9	NS
hTG	µg/l	17.8±9.8	9.7±7.2	$p < 0.05$	10.2±2.4	NS
a-hTG	µg/l	0.7±0.3	0.5±0.3	NS	0.9±0.4	NS
GH	µg/l	3.7±2.2	4.0±2.1	NS	3.9±1.1	NS
Cortisol	nmol/l	570±140	720±130	$p < 0.01$	490±300	NS
PRL	µg/l	10.6±5.7	24.7±14.4	$p < 0.01$	6.9±3.9	NS
PTH	U/l	1.6±0.8	27.5±8.2	$p < 0.001$	3.2±3.0	NS

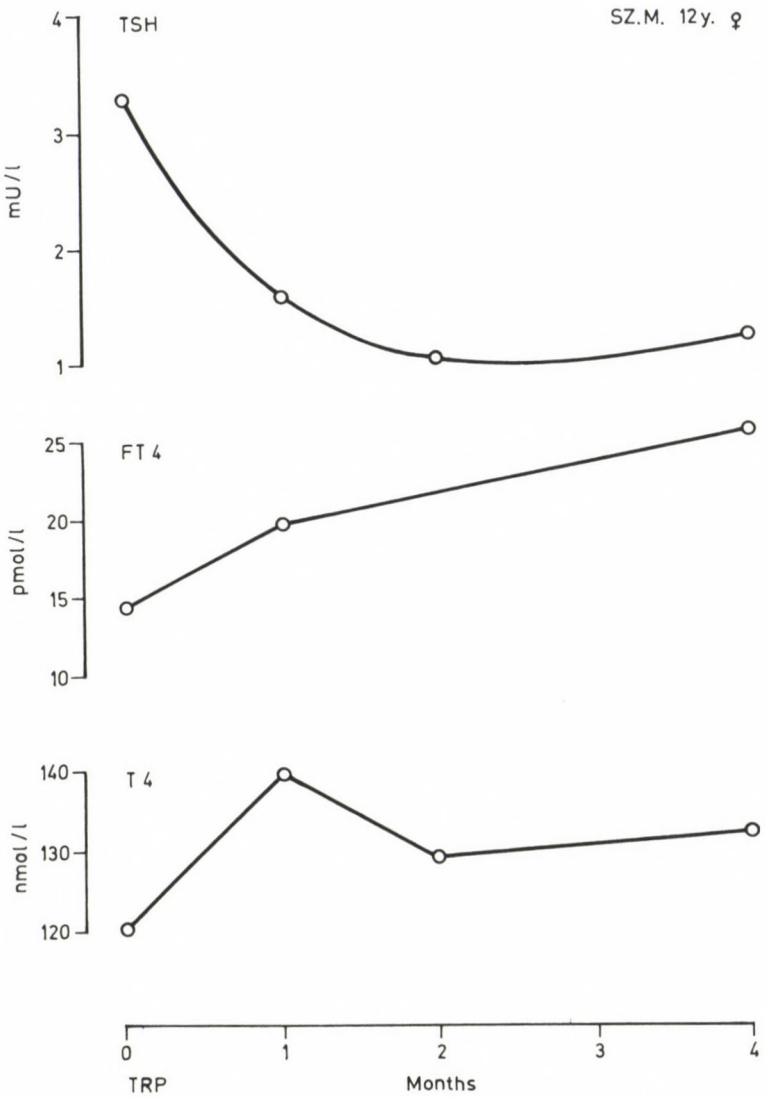

Fig. 1. The change of TSH, FT4 and T4 levels after kidney transplantation

and PTH concentrations were significantly higher in haemodialysed children than in the controls or transplanted subjects. Hyperprolactinemia and defective regulation of PRL secretion have been shown in chronic renal failure, but after a succesful transplantation the serum level of PRL decreased /11/. Figure 2 shows the change of GH, PRL and PTH levels in

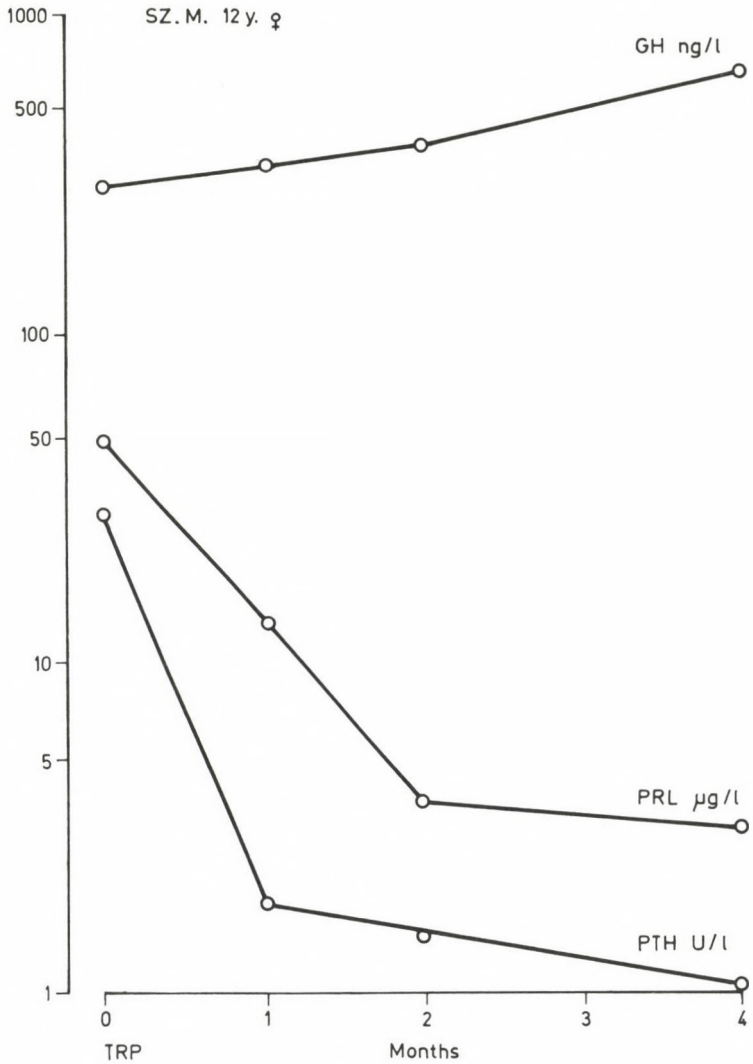

Fig. 2. The change of GH, PRL and PTH levels after kidney transplantation

case of a kidney transplanted girl.

Phosphate retention plays an important role in the pathogenesis of secondary hyperparathyroidism and renal osteodystrophies /23, 19/. We found significant correlation between serum inorganic phosphate concentration and PTH levels,

however there was no correlation with serum calcium concentrations /Figure 3/. The measured and calculated

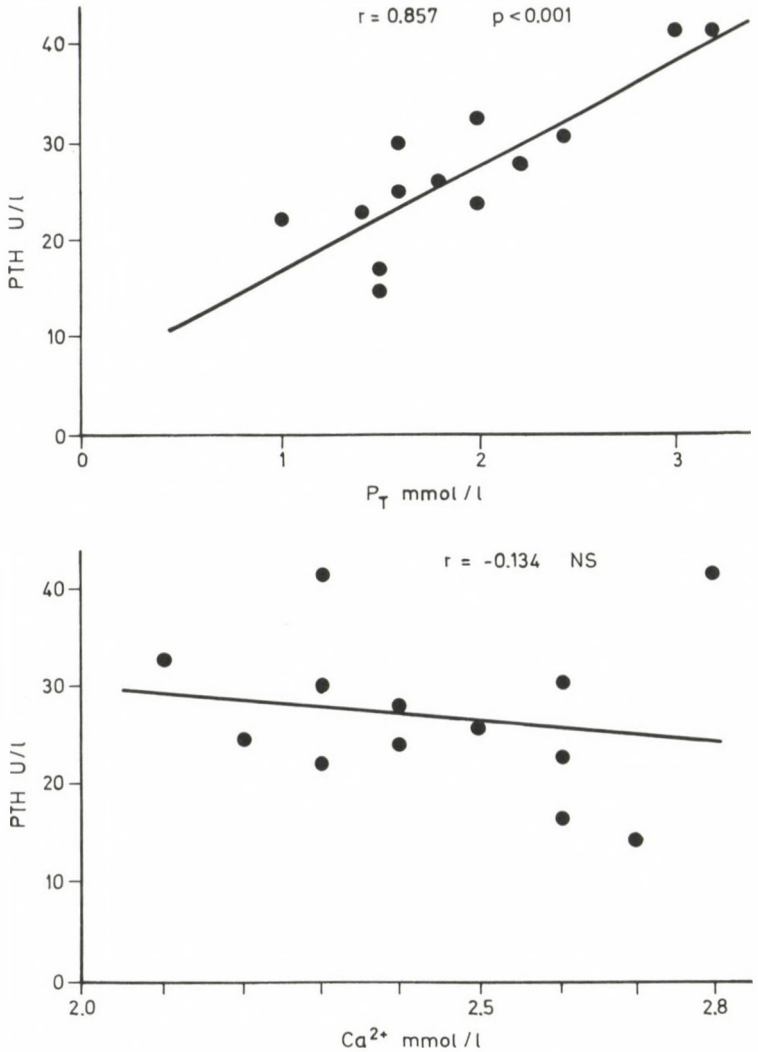

Fig. 3. Correlation between serum inorganic phosphate/calcium concentration and PRH levels

concentrations of pre- and post-dialysis serum samples are listed in Table II. The measured concentrations, with the exception of magnesium, show significant differences.

TABLE II

Results of measured and calculated parameters (mean ± SD)

Serum parameters		Control children	Haemodialysed children pre-dialysis	Haemodialysed children post-dialysis	Transplaanted children
Measured					
Creatinine	µmol/l	81± 12	783± 72^{+++}	344±100^{+++}	120 ± 19^{+++}
Urea	mmol/l	5.5± 1.2	28± 10^{+++}	12± 4^{+++}	7.2 ± 3.0
Phosphate	mmol/l	1.2± 0.3	2.1± 0.6^{++}	0.9± 0.3^{+}	1.3± 0.3
Sodium	mmol/l	142± 3	135± 5^{+++}	136± 4^{+++}	139± 4
Potassium	mmol/l	4.2± 0.3	5.4± 0.8^{+++}	3.7± 0.4^{++}	4.5± 0.2^{+}
Calcium	mmol/l	2.3± 0.1	2.5± 0.3^{+}	2.9± 0.2^{+++}	2.3± 0.1
Magnesium	mmol/l	1.0± 0.1	1.0± 0.2	1.1± 0.2	1.0± 0.2
pH		7.41± 0.05	7.30± 0.06^{+++}	7.44± 0.04	7.36± 0.06
Calculated					
HPO^{2-}_4	mmol/l	0.450±0.145	0.755±0.240^{+++}	0.323±0.108^{+}	0.484±0.151
$H_2PO^{-}_4$	mmol/l	0.138±0.045	0.298±0.100^{+++}	0.092±0.030^{++}	0.164±0.050
$NaHPO^{-}_4$	mmol/l	0.249±0.080	0.398±0.110^{+++}	0.171±0.061^{+}	0.262±0.082
$KHPO^{-}_4$	mmol/l	0.005±0.002	0.012±0.004^{+++}	0.003±0.002^{+}	0.006±0.002
$CaHPO_4$	mmol/l	0.260±0.080	0.473±0.141^{+++}	0.235±0.042	0.278±0.088
$MgHPO_4$	mmol/l	0.093±0.030	0.157±0.055^{+++}	0.072±0.021	0.100±0.032
$\measuredangle CaHPO_4$	%	21.7± 4.6	22.5± 3.9	26.1± 4.2^{+}	21.4± 5.1

$^{+}p < 0.05$ $^{++}p < 0.01$ $^{+++}p < 0.001$ vs. control group

Concentrations of HPO^{2-}_4, $H_2PO^-_4$, $NaHPO^-_4$, $KHPO^-_4$, $CaHPO_4$ and $MgHPO_4$ considerably reduced after dialysis. There was a significant increase in the molar fraction of $CaHPO_4$ (α_{CaHPO_4}) in post dialysis serum, probably for the following reasons:

a. Due to the calcium ion concentration of the dialysis bath, the calcium concentration of the serum increased.

b. The dialysis rate of $CaHPO_4$ complex is different than other phosphate complexes.

Correlation between the effect of dialysis $\left(1-\frac{\text{Post conc.}}{\text{Pre conc.}}\right) \times 100$ and the molecular weight of the analysed constituents is shown of Figure 4. There is a significant negative correlation between the mass of the molecula or ion (X) and the effect of dialysis (Y). The effect of dialysis is the least on the $CaHPO_4$ complex among the different inorganic phosphate complexes. This may play a role in peripherical ischemic necrosis and vascular calcification in chronic renal failure patients.

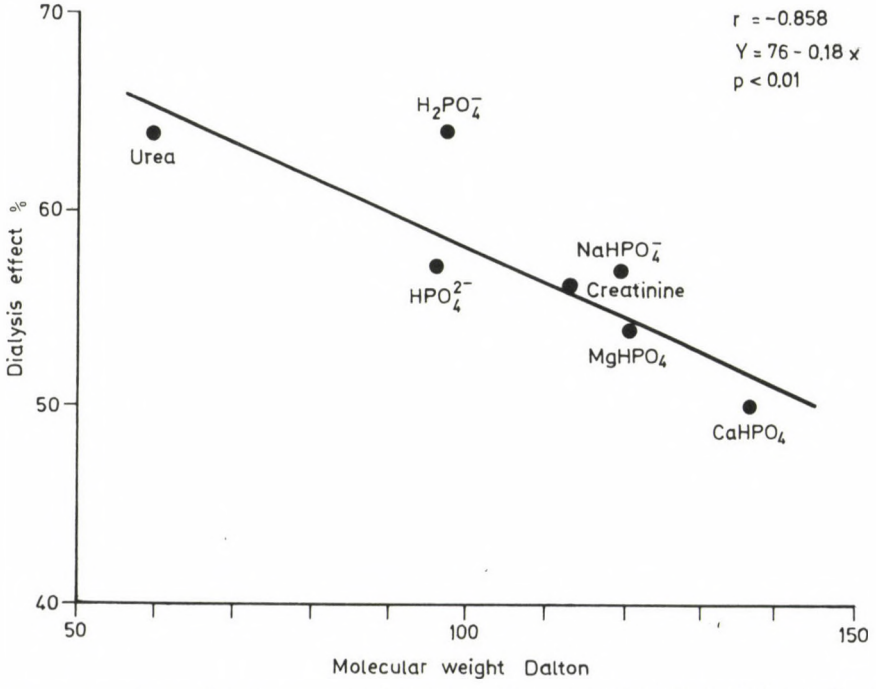

Fig. 4. Correlation between the molecular weight of urea, creatinine, phosphate complexes and dialysis effect.

TABLE III

Results of serum enzyme activities (mean ± SD)

Serum parameters		Control children	Haemodialysed children	Significance vs.control	Transplanted children	Significance vs.control
ACE	U/l	260 ± 106	344 ± 108	NS	553 ± 161	$p < 0.001$
AP	"	92 ± 42	63 ± 41	NS	232 ± 84	$p < 0.001$
Amylase	"	175 ± 51	357 ± 157	$p < 0.001$	317 ± 183	$p < 0.05$
Lipase	"	94 ± 45	177 ± 48	$p < 0.001$	138 ± 91	NS
Ψ-ChE	kU/l	5.4 ± 1.7	4.1 ± 1.5	NS	4.7 ± 0.9	NS
CK	U/l	34 ± 20	49 ± 45	NS	26 ± 11	NS
GOT	"	10 ± 5	9 ± 7	NS	14 ± 8	NS
GPT	"	11 ± 6	8 ± 7	NS	18 ± 13	NS
γ-GT	"	13 ± 7	16 ± 15	NS	17 ± 7	NS
LDH	"	170 ± 30	187 ± 43	NS	192 ± 42	NS

Table III shows the results of serum enzyme activity measurement. Marked differences were noted in the amylase, lipase, ACE and AP activities. In haemodialysed children serum amylase and lipase activity were elevated, while in transplanted children AP and ACE were higher when compared to the controls.

DISCUSSION

Many abnormalities of endocrine function are common due to uremia. The effect of these endocrinopathies on the patient with renal failure ranges from trivial to major /4, 9, 5/. Pathogenic mechanism of endocrine abnormalities in chronic renal failure may be: increased hormone levels by impaired degradation (insulin, glucagon, calcitonin, GH, PTH, PRL) or increased secretion (PTH, LH, FSH, PRL, ACTH) and decreased hormone level by reduced secretion (erythropoietin, $1,25/OH/_2$-D3, gonadal steroids) or impaired conversion (T4, 25/OH/-D3.

Abnormalities of thyroid function tests are frequent in uremic patients /24, 17/. However, relationship between such measurements and clinical thyroid disease is difficult to evaluate because of similarities of some symptoms between the uremic syndrome and hyper- and/or hypothyroidism. While the study of thyroid function in these patients has been hampered by methodologic variation, examination of different parameters, heterogeneity of patient groups, and varying treatment protocols /2/. Our haemodialysed children have serum thyroid hormone levels similar to those described in "sick euthyroid" patients /17, 3/, i.e. low T3, T4 and normal TSH. The most investigators have noted a blunted response of TSH to TRH stimulation /7, 1/. We found the thyroid hormone concentrations at the kidney-transplanted children normalised.

Uremic patients have normal circadian rhythms and normal or slightly elevated level of cortisol /14/. There was a mild increase in cortisol concentration in our haemodialysed

children ("stress effects"). Growth hormone concentration is commonly elevated in chronic renal failure, decreased renal excretion of the hormone is the major cause of its elevation /20, 15/. Opposite to literature data, in our study we did not find significant change in GH level, but growth retardation was evident in children on haemodialysis, suggesting the presence of end-organ resistance to the action of the hormone. The levels of PRL hormone are elevated in the majority of patients with renal failure. Hyperprolactinemia results from both increased secretion and reduced degradation /11, 9, 22/.

Parathyroid hormone may have actions in immunological responses, carbohydrate-, lipid-metabolism, bone marrow fibrosis, sexual potency and erythropoiesis. PTH has numerous direct action on the kidney, where it is the primary regulator of renal phosphate homeostasis /2/. PTH may effect serum phosphate concentration, depending upon the degree of renal insufficiency. With mild to moderate renal impairment, increased secretion of PTH can lower the renal tubule phosphate absorption and reduce serum phosphate. As the renal insufficiency progresses, the renal capacity to reabsorb phosphate is reduced despite the very high level of PTH. Moreover, PTH acts on bone to enhance osteoclastic resorption, which increases the release of phosphate and calcium into the blood. We found significant correlation between serum phosphate concentration and PTH level, however there was no correlation with calcium concentration (Figure 3). Lopez-Hilker et al also documented that hypocalcemia may not be essential for the development of secondary hyperparathyroidism in chronic renal failure /12/. Massry and Eberhard have shown an interrelationship between phosphate retention, skeletal resistance to PTH, hypocalcemia, and secondary hyperparathyroidism in patients with advanced renal failure /13/. A possible role of hyperphosphatemia and phosphate retention in accelerating or aggravating the progression of renal damage has been suggested from observations in experimental animals /10/.

In chronic renal failure, among multiple factors, the physicochemical complexation of phosphate and calcium and

impaired gastrointestinal absorption alter the calcium metabolism. Resulting syndromes of tissue necrosis, vascular calcification and osteodystrophies were reported /19. 8/. The corresponding damage to the various organs in the most cases is irreversible. Diet, phosphate binding antacids may prevent or delay the above processes and phosphate free dialysis fluid should be used. From other side the complex treatment sometimes overcompensates to the extent that it results in hypophosphatemia which may cause uremic bone dystrophy /16/.

Presently, there are no practical analytical techniques available for the quantitation of the individual inorganic phosphate constituents, mainly due to the unavoidable alteration of the physiological equilibrium during the various analytical procedures /21/. Numerical data are reported in our study from $CaHPO_4$ which plays the major role in all kinds of calcification. However, it confirms the fact that to emphasize and distinguish the role of calcium and phosphate ions separately is not advisable in uremic state and beside the endocrine regulation there are complex-chemical interactions, too, one has to reckon with.

Angiotensin I converting enzyme (ACE) is a membrane-bound glycoprotein, primarily localized in the endothelial cells of the pulmonary capillaries and the kidney cortex. Some pathological conditions can be responsible for increased activities of ACE /25/. The elevation of ACE observed in our transplanted children may be due to the relative narrowing of the vascular anastomosis. Although total serum alkaline phosphatase (AP) includes isoenzymes arising from liver, intestine, kidney and bone, its measurement can be a useful indicator of increased osteoblastic activity. The high elevated serum AP was observed in transplanted children as a signal of the catch-up growth which occurred in most patients after grafting. The enzyme amylase is a 50 000 dalton protein derived predominantly from salivary glands and pancreas. Amylase is readily filtered at the renal glomerulus and may be partially reabsorbed in the tubule. In renal insufficiency, therefore, it is common to observe a mild to moderate elevation of serum amylase activity /18/. Pancreas-specific enzyme values,

including lipase activity and trypsin concentration, are also found to be nonspecifically elevated in the presence of renal insufficiency /6/. There were no alterations in the further investigated enzymes (Ψ-ChE, CK, GOT, GPT, γ-GT, LDH) compared to the control group. In the catabolism of these enzymes the kidney does not play any important role.

Comprehensively it can be stated the examination of the hormones, of the changes in calcium-phosphate concentration and of the enzyme activities in end state renal disease may provide further informations on the complex metabolism disturbances. The normalisation of several investigated parameters following kidney-transplantation is supporting the fact that the kidney plays important role in these processes.

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