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Secondary Hyperparathyroidism in Rats with Experimentally Induced Pyelonephritis*

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Summary. The influence of induced pyelonephritis without uraemia on the function and morphology of the parathyroids was studied in the rat. Animals with induced pyelonephritis of the left kidney and simultaneous right nephrectomy were compared with animals with induced pyelonephritis of the left kidney and the right kidney left intact. Untreated animals served as controls. - The kidney function was evaluated by the serum creatinine and the polyethylene glycol clearance (GFR). Eight weeks after induction of pyelonephritis no obvious impairment of the total kidney function was observed in the pyelonephritis group without nephrectomy and only moderate impairment was found in the nephrectomized group. In both groups quantitative estimations showed that the induced pyelonephritis was accompanied by parathyroid hypertrophy and hyperplasia. Ultrastructural studies indicated a similar degree of enhancement of the parathyroid activity in the two experimental groups. The more pronounced renal dysfunction in the group with the right kidney removed was not accompanied by a higher degree of hypertrophy and hyperplasia of the parathyroids. - Our findings support the concept that in man also secondary hyperparathyroidism may develop in the presence of only slight or moderate impairment of the renal function.

Key words: Secondary hyperparathyroidism, experimental pyelonephritis, morphology

The pathogenesis of secondary hyperparathyroidism in chronic renal disease (21) still remains unknown. O'Riordan et al. (20) could not find any correlation between the plasma concentrations of calcium and parathyroid hormone in untreated patients with chronic renal disease.

Hypocalcaemia has generally been considered to be the most important causative factor in secondary parathyroid hyperplasia (1). However, Bergdahl & Boquist (2) state that hypocalcaemia is of less importance than previously believed in the development of human secondary hyperparathyroidism. Some factors in the kidneys seem to play a role in this respect. It has been suggested that the increased serum levels of parathyroid hormone may directly contribute to a resistance to (7) or a defect in the metabolism of vitamin D (18) in chronic renal failure.

The present report deals with studies on the association between experimentally induced pyelonephritis and secondary hyperparathyroidism. The rat was chosen as the experimental animal because enlargement of the parathyroid glands in chronic experimental renal insufficiency has been reported in this species (10, 11, 12, 17, 19, 30), and the aim of this investigation was to study the influence of slight to moderate pyelonephritis without uraemia on the function and morphology of the parathyroids.

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Material and Methods

Thirty-three adult male rats of the Sprague-Dawley strain, weighing 250-300 g, were used. During the experimental period they were fed on laboratory food containing 0.8% calcium and 0.6% phosphate and had free access to water. The animals were divided at random into the following groups:

1. 12 control rats - untreated;
2. 11 rats with experimentally induced pyelonephritis of the left kidney and with the right intact (PN-I);
3. 10 rats with experimentally induced pyelonephritis of the left kidney and simultaneous right nephrectomy (PN-II).

Pyelonephritis was induced by the method of Shankel et al. (29) as modified by Fredriksson et al. (6). The left kidney was exposed through a

midline abdominal incision and the parenchyma was punched 100 times with a needle repeatedly dipped into a culture of enterococci (10^{10}/ml).

The animals were sacrificed 8 weeks after the induction of pyelonephritis.

Laboratory Methods

The functional state of the parathyroids was examined by determining the serum levels of calcium and phosphate, and the renal function was assessed by estimating the serum creatinine and the glomerular filtration rate (GFR) per 100 g body weight. GFR was estimated as clearance of polyethylene glycol, molecular weight 800-1000 (PEG 1000) under barbiturate anaesthesia; PEG_{1000} was administered by constant intravenous infusion and urine sampling took place through ureteral catheters.

Light Microscopy

The larynx together with the thyroid and parathyroid glands was removed from all but 8 rats and fixed in 10% formalin. In one control animal the fixation was performed by perfusion of 10% formalin in vivo. The parathyroid glands of this animal were not used for the quantitative studies described below. The paraffin-embedded specimen was sectioned throughout in series of three consecutive 5 µ thick sections with intervals of 50 µ between each series. Two sections in each series were stained with haematoxylin-eosin or Van Gieson's stain. The third section was stained either by an argyrophil method (8) or with PAS.

The kidneys from the different rats were fixed in 10% formalin, and 5 µ thick deparaffinized haematoxylin-eosin stained sections were examined.

Electron Microscopy

Exised parathyroid glands from 7 rats - 3 controls, 1 from group PN-I and 3 from group PN-II - were fixed in 2.5% glutaraldehyde in a 0.1 M phosphate buffer at pH 7.4 for 4 h at 4° C. The tissue pieces were post-fixed in 2% osmium tetroxide in a 0.1 M phosphate buffer at pH 7.4 for 2 h at 4° C. The fixed tissue was then dehydrated and embedded in Epon. Ultrathin sections, cut with an LKB Ultrotome, were contrasted with uranyl acetate followed by lead citrate and examined in a Zeiss electron microscope (EM 8).

Quantitative Estimations

Volume estimations of the parathyroid glands.[1] The serial haematoxylin-eosin stained sections were projected with a Leitz microprojector by means of a mirror onto a table at a magnification of x 300. The contours of the parathyroid glands were drawn on paper and the area was estimated planimetrically. The total area in each case was multiplied by the interval between the adjacent haematoxylin-eosin sections (= 60 µ).

Cellular density. The density of parenchymal cells in the parathyroid glands was determined by counting the number of nuclei and nuclear fragments within a unit volume, in a light microscope at a magnification of x 1000. In one of the eyepieces a squared grid, which covered an area of 0.0169 mm^2 of the section at the above mentioned magnification, was placed. The section was 0.005 mm thick and the determined volume was 8.45×10^{-5} mm^3 (unit volume). Five randomly selected unit volumes from the largest serial section were estimated. Sections from 5 cases from each group were included in this investigation.

In counting the particles in a section, the number of observed particles is influenced both by the size of the particles and by the section thickness (4, 9). Particles with a large diameter are over represented as compared with smaller ones. Using the formula of Floderus (9), the obtained frequency values are corrected and only the number of particles whose centres lay within the sections was obtained.

Our values were corrected by means of the above formula. The section thickness was the same in the different groups and the smallest nuclear fragment measured had a diameter of about 1 µ. From the cell density (corrected values) and gland volume, the total number of parenchymal cells in the parathyroid glands was calculated.

Nuclear size. From 15 animals (5 of each group) the largest parathyroid serial section, stained with haematoxylin-eosin, was photographed in black and white. On the photographs, which had a final magnification of x 1850, the surface areas of 400 randomly selected parenchymal cell nuclei were calculated[2] with the aid of a particle area analyser (Zeiss TGZ 3).

In 6 rats the nuclear areas of 200 dark and 200 light chief cells were calculated as above.

The error of the method was calculated by duplicate estimations of the areas of 200 nuclei randomly selected from each of 6 animals.

The error of the sampling was calculated in 6 rats by estimating the areas of 100 nuclei from each of 4 randomly selected regions.

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1 This was kindly performed by Miss Lena Johansson

2 This was kindly performed by Hans Johansson

Concerning the calculations of the error of method and sampling, see Erenkö (3).

Results

Body Weight

No significant differences in body weight at sacrifice were found between the animals of the three groups (Table 1).

Table 1. Body weight at sacrifice

	No. of animals	Body weight (g) M ± SE
Controls	12	448 ± 7.1
PN-I	11	457 ± 7.1
PN-II	10	454 ± 10.0

Kidney

Weight and function. The total kidney weight at sacrifice is given in Table 2. In group PN-I the left diseased kidney weighed 66 ± 3.0 (S.E.) % of the weight of the right kidney. In group PN-II the single diseased kidney (left) weighed about the same as both kidneys together in the controls and the animals of group PN-I.

The serum creatinine level (Table 2) was significantly increased after 8 weeks in group PN-II in comparison with both the control group ($p < 0.001$) and group PN-I ($p < 0.001$), but in all groups it lay within the normal range of our laboratory method. (M = 0.76 mg/100 ml, SD = 0.14, n = 103).

GFR was significantly lowered in group PN-II ($p < 0.001$) but not in group PN-I, in comparison with the controls (Table 2).

Gross anatomic findings. The kidneys of the control group and the initially excised right kidneys of group PN-II appeared normal on gross examination. In group PN-I the left kidneys were slightly diminished and showed irregular scarred surfaces, while the right kidneys were slightly enlarged. In group PN-II the remaining kidneys were irregularly shaped, considerably enlarged, and showed a greyish discoloration. No spots of suppuration were seen on the renal cortex.

Light microscopic findings. In the control group the kidneys of 8 animals were examined. In 5 of the animals no signs of inflammatory reaction were found. In one animal slight subacute pyelitis was seen bilaterally, while in 2 animals one kidney contained small solitary infiltrates of inflammatory cells, mainly lymphocytes, often located perivascularly in the cortex. In these kidneys no fibrosis or areas of granulation tissue were observed.

In group PN-I all animals showed pyelonephritis in their left kidney; in addition, 4 animals displayed slight pyelonephritis and another 4 animals slight pyelitis in their right kidney. In group PN-II pyelonephritis was found in the remaining kidney in all animals.

The inflammatory cells were mainly lymphocytes, but granulocytes and histiocytes were also seen.

Parathyroid Glands

Function. The serum calcium and phosphorus levels are given in Table 3. After an experimental period of 4 weeks no differences in the serum calcium level between the groups were found. After 8 weeks group PN-II showed a mean serum calcium level significantly lower than that of both the controls ($p < 0.05$) and group PN-I (p 0.001). After 8 weeks the serum phosphorus level in the experimental groups did not differ from that in the control groups.

Volume. The volume of the parathyroid glands was significantly increased in the two experimental

Table 2. The weight and function of the kidney

	No. of animals	Total kidney weight (g) at sacrifice M ± SE	Serum creatinine (mg/100 ml) M ± SE Weeks after induction of pyelonephritis		Glomerular filtration rate/100 g body weight M ± SE Weeks after induction of pyelonephritis
			4	8	8
Controls	12	3.7 ± 0.2	0.67 ± 0.01	0.67 ± 0.06	0.84 ± 0.06
PN-I	11	3.5 ± 0.1	0.62 ± 0.02	0.69 ± 0.02	0.74 ± 0.07
PN-II	10	3.3 ± 0.3	0.79 ± 0.02	0.99 ± 0.07	0.28 ± 0.04

Table 3. Serum and phosphorus

	No. of animals	Serum calcium mEg/L M ± SE Weeks after induction of pyelonephritis			Serum phosphorus mEg/L M ± SE Weeks after induction of pyelonephritis
		0	4	8	8
Controls	7	5.3 ± 0.03	5.0 ± 0.02	4.8 ± 0.15	4.3 ± 0.10
PN-I	10	5.3 ± 0.03	4.9 ± 0.05	5.1 ± 0.03	4.1 ± 0.21
PN-II	7	5.3 ± 0.04	4.9 ± 0.03	4.1 ± 0.22	4.4 ± 0.26

Table 4. Mean volume (in mm^3) of rat parathyroid glands

	No. of animals	Absolute volume M ± SE	P values (t-test) for experimental groups compared with the control group	Volume/100 g body weight M ± SE	P values (t-test) for experimental groups compared with the control group
Controls	7	0.293 ± 0.012		0.067 ± 0.003	
PN-I	10	0.462 ± 0.020	<0.001	0.101 ± 0.004	<0.001
PN-II	7	0.439 ± 0.035	<0.01	0.097 ± 0.008	<0.01

groups as compared with the control group (Table 4). The volume of the glands in group PN-I was somewhat, but not significantly increased compared with group PN-II.

Light microscopic findings. In all rats two parathyroid glands were found. The glands were surrounded by a thin capsule.

The parenchymal cells were often arranged in nests or cords surrounded by capillaries or thin strands of connective tissue. In some areas the parenchymal cells were arranged in solid sheets.

As regards the cell arrangement, vascularity and amount of connective tissue, there were apparently no differences between the glands of the experimental and control groups.

The glands contained dark and light chief cells (Fig. 1 and 2). The light cells appeared to be somewhat larger than the dark type and showed a light or an apparently clear cytoplasm. The perfusion fixed glands also contained light parenchymal cells, indicating that these cells are not fixation artefacts. The parenchymal cell nuclei were often more hyperchromatic and more irregular in shape in the experimental rats than in the controls.

In the control group the frequency of light cells varied between 10 and 30%. In group PN-I this frequency was increased and in some animals comprised 40-45% of the parenchymal cells. In group PN-II the frequency of light cells was somewhat lower than in group PN-I, but slightly increased compared with the control group.

No PAS-positive or argyrophil reaction was seen in the parenchymal cells either in the control rats or in the experimental groups.

Electron microscopic findings. The ultrastructural findings (Fig. 3 and 4) were essentially similar in groups PN-I and PN-II, but compared with the controls these two groups showed an enlarged Golgi complex, an increased number of ribosomes and accentuated tortousity of the cell membrane. The rough-surfaced endoplasmic reticulum appeared rather flat in the experimental groups. The secretory granulas were few in number both in experimental and control animals. The light chief cells, which occurred in a large number in the experimental animals, contained a less dense cytoplasmic matrix than the dark cell type.

Quantitative Estimations

Cellular density. The cellular density is shown in Table 5. In both experimental groups there was

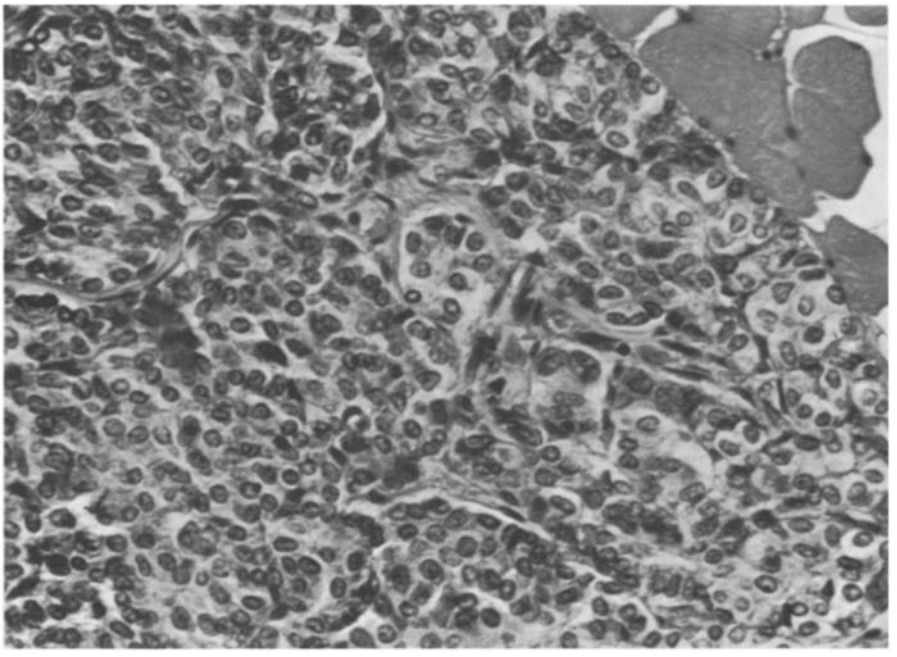


Fig. 1. Parathyroid gland of a control rat. Dark chief cells are predominant. x 450

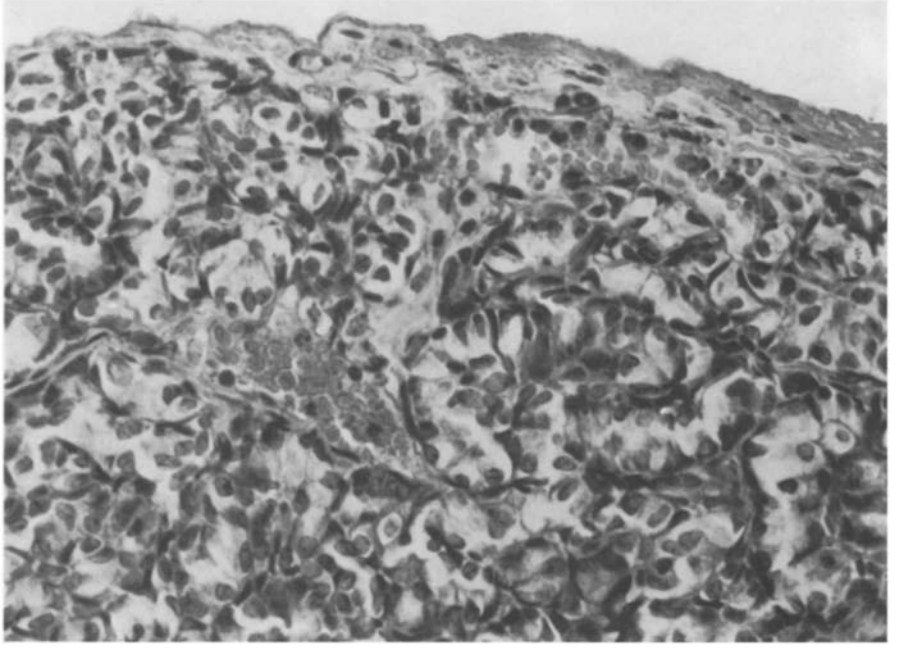
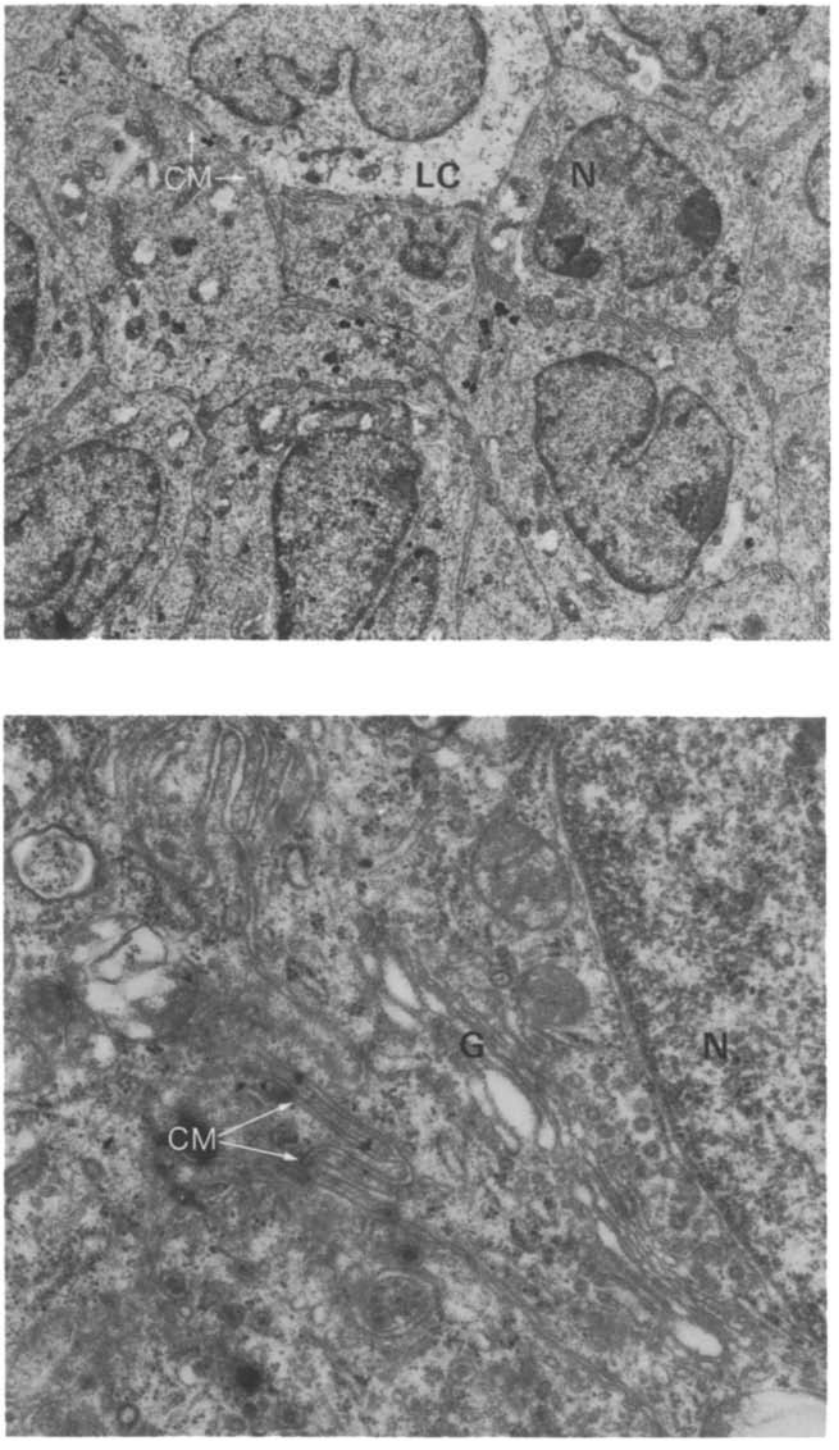


Fig. 2. Parathyroid gland of a rat of group PN-I. The light chief cells are increased in number. The nuclei are often more hyperchromatic and irregular in shape than in control rats. x 450



Figs. 3 and 4. Ultrastructural picture of parathyroid cells from a control (Fig. 3) and an animal of group PN-I (Fig. 4). The cellmembrane is less tortuous and the Golgi apparatus less prominent in the control than in the experimental animal. LC = Light chief cell, N = Nucleus, CM = Cell membrane, G = Golgi apparatus

a significantly lower frequency of parenchymal cells per volume unit than in the control group.

The total number of parenchymal cells in the parathyroid glands (Table 6) was significantly increased in group PN-I compared with the controls. There was also an increase in group PN-II, but this was not significant.

Nuclear size. The mean diameters of the parenchymal cell nuclei in the different groups are given in Table 7. The nuclear diameter was increased in both experimental groups compared with the controls but only in group PN-I was the increase significant.

No difference in nuclear size was seen between the dark and light chief cells (t-value = 1.23).

The error of the method and error of sampling were small, 0.023 and 0.110 μ, respectively, and no greater in the experimental groups than in the controls.

Table 5. The observed and corrected (using Floderus' formula) number of nuclei and nuclear fragments per 5 x unit volumes (unit volume = 8.45×10^{-5} mm^3). The corrected values represent the number of nuclear centres within unit volumes

	No. of animals	Observed No. of nuclei and nuclear fragments $M \pm SE$	Corrected No. of nuclei and nuclear fragments $M \pm SE$	P values (t-test) for experimental groups compared with the control group
Controls	5	1.275 ± 36	659 ± 26	
PN-I	5	1.096 ± 60	539 ± 28	< 0.02
PN-II	5	1.102 ± 54	552 ± 29	< 0.05

Table 6. Total number of parenchymal cells in the parathyroid glands

	No. of animals	Total No. of parenchymal cells $M \pm SE$	P value (t-test) from experimental groups compared with the control group
Controls	5	$(33.41 \pm 2.36) \times 10^4$	
PN-I	5	$(46.07 \pm 0.54) \times 10^4$	< 0.01
PN-II	5	$(42.56 \pm 4.78) \times 10^4$	< 0.20

Table 7. Diameters (μ) of parathyroid cell nuclei calculated from photographs by a particle area analyser. The diameters of 400 randomly selected nuclei were estimated in the largest serial section in each rat

	No. of animals	Nuclear diameter (μ) $M \pm SE$	P value (t-test) from experimental groups compared with the control group
Controls	5	4.79 ± 0.13	
PN-I	5	5.25 ± 0.13	0.05
PN-II	5	5.10 ± 0.13	0.20

Discussion

The method used to induce pyelonephritis is a modification of that described by Shankel et al. (29) and has been practised in recent studies from this laboratory (6). Light microscopic examination at the end of the experimental period showed a clear inflammatory reaction in all the left kidneys of groups PN-I and PN-II. A slight inflammatory reaction was also found in some of the right kidneys of group PN-I as well as in some of the kidneys of the control animals, but to a much lesser degree. Moreover, the kidney function measured by GFR was significantly decreased only in group PN-II, and in this group the amount of renal parenchyma was sufficient not to give laboratory evidence of uraemia. In group PN-I the GFR of the left kidney was about 65% of that of the right side. The pyelonephritis produced by the method described would therefore seem to be a valuable model for studying the pathophysiology of the parathyroid glands in chronic renal disease without uraemia.

The volumetric estimation disclosed a significant enlargement of the parathyroid glands in the experimental groups, especially in PN-I. The cell density per unit volume was decreased in both experimental groups, indicating a cell hypertrophy. Further, our rough estimations of the total number of parenchymal cells showed a higher cell frequency in the experimental animals than in the controls, i.e. a hyperplasia is added to the above mentioned hypertrophy.

The increase in nuclear size in the experimental groups, as well as the ultrastructural findings of an enlarged Golgi complex, an increased number of ribosomes and increased tortuosity of the cell membrane indicated an enhancement of the parathyroid cell activity (14, 23, 26). Thus, the combined quantitative and qualitative morphological alterations clearly showed that the experimentally induced pyelonephritis without uraemia was accompanied by parathyroid hypertrophy and hyperplasia.

An increased number of light chief cells observed in our experimental groups has also been reported previously in animals with renal insufficiency (14). However, no significant difference was noted between the nuclear size in light and dark chief cells, indicating that there was no difference in cellular activity. The reason for the increased frequency of light cells is unclear, but it may reflect a chronically stimulated cell or a cell undergoing restitution.

Similar morphological changes and enlargement of the parathyroid glands were demonstrated by Shimamura & Morrison (30) in rats with resection of 5/6 of the kidney, but these alterations did not occur until the time when the rats became uraemic. In their studies, however, the observed changes in the cells of the enlarged parathyroid glands were of the same nature as those seen by others in stimulated glands in vivo (23) and in vitro (25, 26) and those occurring in renal disease in humans (24). Our experimental findings indicate that hyperplastic and hypertrophic changes can be induced in the parathyroids by renal injury, even without uraemia. This is of interest, as it has been stated by Bergdahl & Boquist (2) that secondary hyperparathyroidism may develop in subjects with only slight or moderate impairment of renal function.

Only the total serum calcium was determined in the present study. Ionic calcium is the physiologically active component and seems to be especially useful to measure in subjects whose total calcium values fluctuate within the normal range (15). Furthermore, the degree of ionization may be higher in renal acidosis. The mean serum calcium level was different in the two experimental groups. In group PN-I it was unaffected, in accordance with earlier reports (12, 17), whereas in the group with the intact kidney removed (PN-II) obvious hypocalcaemia was seen. In rabbits, Rutishauser (27) observed hypercalcaemia 90 and 138 days after the development of experimental nephritis, i.e. a reverse effect compared with that seen in th present study.

It is generally considered that the serum calcium level is low or normal in secondary hyperparathyroidism and that hypocalcaemia, as was found in one of our experimental groups (PN-II), may be a stimulus to the development of parathyroid hyperfunction. The findings in our other experimental group (PN-I), however, indicated that secondary parathyroid hyperplasia and hyperfunction may develop in the absence of visible hypocalcaemia, which is consistent with observations in dogs with secondary hyperparathyroidism (2). However, in view of the complex interactions between homeostatic mechanisms it is conceivable that phosphate retention or a disturbance of vitamin D metabolism may act as a hypocalcaemic factor but that a parathyroid hormone secretion response maintains normal serum levels of calcium and phosphorus (32).

There is evidence that the kidney can be regarded as an endocrine organ with an important role in regulating calcium, phosphorus and bone metabolism (5, 31), which may be relevant to our finding that under certain conditions hyperplasia of the parathyroid glands may develop early during the course of kidney disease. However, the possibility that the parathyroid activity is inadequately stimulated by renal factors, and attempts by the parathyroid glands to compensate for a disturbed vitamin D metabolism or phosphorus retention might be considered as causative mechanisms (13).

The more severe renal dysfunction in group PN-II was not accompanied by a more marked degree of parathyroid hyperplasia than in group PN-I. In group PN-II the tubular reabsorption of phosphate must have been much lower than in PN-I, to judge from the values of GFR and serum phosphate. However, this does not necessarily imply a higher leve of parathyroid hormone in group PN-II, as a lower

GFR may in itself cause an increased fractional reabsorption of water and phosphate (28). As the kidneys are important for the metabolism of parathyroid hormone (16), its elimination from the blood was probably slower in group PN-II than in the controls and in group PN-I. This means that even in the presence of the same degree of parathyroid hyperplasia and with the same rate of secretion of parathyroid hormone, group PN-II could well have had a higher level of hormone in the serum than group PN-I.

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