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Hyperparathyroidism and Experimental Chronic Renal Failure*

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Summary. Moderate renal failure was produced in 10 dogs by heminephrectomy on one side and contralateral nephrectomy. Hypercalcaemia and hyperphosphataemia developed in all the animals, and there was volumetric, light microscope and ultrastructural evidence of hyperparathyroidism. The hyperplastic parathyroid glands mainly consisted of chief cells with features of great functional activity. The results show that secondary hyperparathyroidism may develop in the absence of hypocalcaemia.

Key words: Parathyroids, hyperparathyroidism, renal failure, quantitative histology

Introduction

Chronic renal disease is the most common cause of secondary hyperparathyroidism, and hypocalcaemia has generally been considered the most important factor in the development of this kind of parathyroid hyperfunction (1). A recent study (2) has suggested, however, that the pathogenetic role of hypocalcaemia in the development of secondary hyperparathyroidism in man may be less important than was formerly believed. Enlargement of the parathyroid glands was reported in rats (10, 18, 19, 21, 24, 28, 29) and dogs (16) with experimental chronic renal lesions, but no reliable information about serum calcium levels was obtained in these studies; marked hypercalcaemia occurred in rabbits with experimental nephritis (32). There is only one previous report of the fine structural alterations of the parathyroid glands in experimental chronic renal disease (34).

The present work is part of a study of the association between renal disease and hyperparathyroidism. The dog was chosen as the experimental animal because hyperparathyroidism secondary to chronic nephritis or renal cortical hypoplasia has been reported in this species (7, 15, 17, 20, 22, 26, 31, 37), and they may even develop primary hyperparathyroidism (13, 14, 23, 30, 36).

Material and Methods

Animals: Ten dogs (6 males and 4 females), aged 6 months to 8 years, and weighing 11 to 34 kg were used. Chondrodystrophic dogs were excluded because their skeletons are abnormal anyway and they have a predisposition to urolithiasis (23). Before the operations the dogs appeared healthy and they had normal renal function. During the experimental period they were fed a standard diet for mink (Vasterbottens Slakteriforening A. B., Umea, Sweden) and had free access to drinking water. The dogs were kept in large cages.

Surgical Technique: The operations were performed in two stages, on each occasion the animals were anaesthetized intravenously with a 6% solution of Pentobarbitalum (Mebumal, ACO, Stockholm) at a dose of 0.5 ml/kg body weight, and were intubated.

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At the first operation, approximately 50% of the left or right kidney was removed through a subcostal incision, and, after thorough hemostasis, the cut surface was covered with perirenal fat tissue. One week later, the remaining kidney was removed, also through a subcostal incision.

After every operation an intramuscular dose of 2 ml of benzylpenicillin procaine, 300 000 I. U. /ml (Suspenin, Kabi, Stockholm) was given. Specimens from the excised kidneys were examined by light microscopy.

There were no postoperative complications. In the postoperative period blood samples were taken every week from superficial limb veins. Renal function and serum calcium and phosphorus concentrations were studied for 5 weeks after the second operation.

The animals were killed approximately five weeks after the second operation. Immediately beforehand, specimens were taken for light and electron microscopy from all the parathyroids. Afterwards, specimens for light microscopy were taken from the remaining renal parenchyma.

Statistical Methods: The data from the laboratory examinations was tested statistically using a computer (Controldata 3 200) and the Method of Repeated Measurements (Morrison, 1967). The volumetric data was also tested statistically, using non-parametric methods (Wilcoxon and Mann-Whitney).

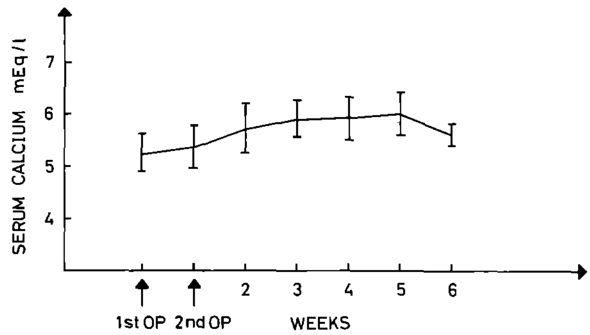

Fig. 1. Mean serum calcium levels in 10 dogs after heminephrectomy on one side (1 st OP) and nephrectomy on the other (2 nd OP). The vertical bars indicate the standard deviation (S. D.).

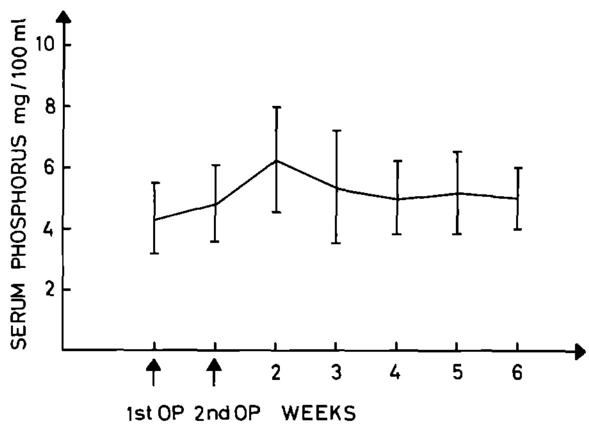

Fig. 2. Mean serum phosphorus levels in 10 dogs after heminephrectomy on one side (1 st OP) and nephrectomy on the other (2 nd OP). The vertical bars indicate the S. D.

Results

Operative Postoperative and Autopsy Findings

No complications occurred during or after the operations. Two superior and two inferior parathyroid glands were identified in all the dogs; the glands seemed to be enlarged. At operation both kidneys were grossly normal. The remaining renal parenchyma appeared to be enlarged at autopsy.

Laboratory Findings

The mean serum calcium level before the operations was 5.2 mEq/1. After the first operation there was a slight, non-significant increase in the serum calcium concentration (Fig. 1). The second operation was followed by a significant ($p<0.01$) rise which tended to fall by the end of the period of observation.

The mean serum phosphorus level before the operations was 4.3 mg/100 ml. After the first operation there was a slight but non-significant increase (Fig. 2), and the second operation was followed by a significant ($p < 0.05$) elevation of the serum phosphorus

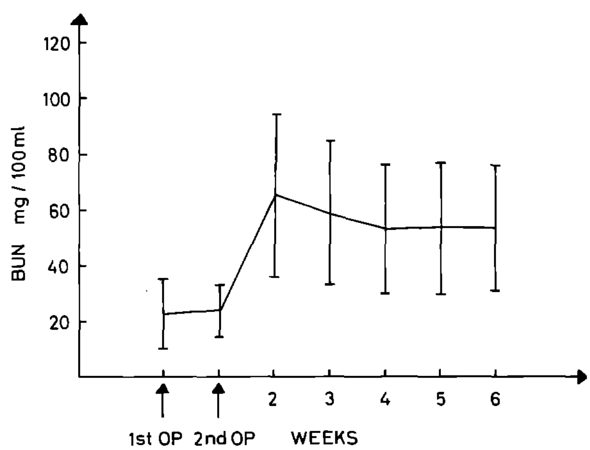

Fig. 3. Mean blood urea nitrogen concentrations in 10 dogs after heminephrectomy on one side (1 st OP) and nephrectomy on the other (2 nd OP). The vertical bars indicate the S. D.

level. The highest mean value (6.3 mg/100 ml) was found one week after the second operation. The standard deviation was higher for phosphorus measurements than for calcium. The product of Ca x P was not constant during the experimental period; during the first weeks it was increased and at the end of the experimental period it was decreased.

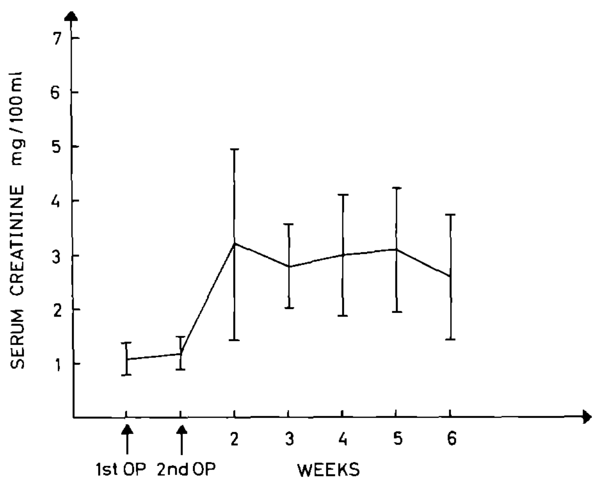

Fig. 4. Mean serum creatinine levels in 10 dogs after heminephrectomy on one side (1 st OP) and nephrectomy on the other (2 nd OP). The vertical bars indicate the S. D.

Renal function, assessed by determination of the mean levels of BUN and serum creatinine, was not altered after the first operation (Figs. 3 and 4), but after the second, a sharp rise was found in the levels of both BUN and serum creatinine. At the end of the experiment there were still laboratory signs of moderate renal failure.

Light Microscopic Findings

The kidneys removed at operation were microscopically normal. Kidneys obtained at autopsy (5 weeks after the second operation) showed features of hyperplasia and the tubular epithelium had partly degenerated.

The morphological alterations in the parathyroid glands were similar in all the dogs. The glands were enlarged as compared with those of normal dogs (3), and they were rounded, oval or egg-shaped. The capsule was unaffected. No fat cells were observed. The parenchyma mainly consisted of light and dark chief cells in solid cords and sheets and oxyphil cells were infrequent. Cellular aggregates composed of syncytial cells and small chief cells were irregularly distributed in the parenchyma. The number of these aggregates seemed to be decreased in some of the glands, as compared with the findings in normal dogs. Follicles were sometimes seen. They were lined by cuboidal or cylindrical cells in parallel arrangement, or by irregularly arranged polygonal cells. The contents of the follicular lumina varied; some were empty, others contained fluid, desquamated epithelial cells or cellular debris.

Volumetric Determinations

The mean number ± S. D. of nuclear hits was 636.4 ± 31.6 after operation, as compared with a mean value of 321.0 ± 4.0 in normal dogs (3). The difference between these values was significant ($p < 0.05$).

The nuclear measurements showed a mean value of the product $D_1 \times D_2$ of 21.1 ± 1.1, as compared with a mean value of 15.6 ± 1.4 in normal dogs. The difference between these values was significant ($p < 0.05$).

Electron Microscopic Findings

Many chief cells showed varying electron density of their cyloplasm and signs of high activity; the Golgi complex was enlarged (Fig. 5), and the endoplasmic reticulum was prominent and often of lamellar type (Fig. 6). Most often only a few secretory granules were found. Some chief cells contained smooth-surfaced or rough-surfaced cytoplasmic vacuoles that were either electron lucent or contained amorphous masses or small vesicles of varying shape (Fig. 7). Glycogen accumulations (Fig. 5), lipoid bodies and a moderate amount of normal-sized or enlarged mitochondria were observed. Occasional mitochondria contained whorls of cristae or tubules in parallel arrays. Cilia and cytoplasmic annulate lamellae were sometimes seen. The cell membranes showed microvilli and sometimes interdigitated with each other.

The few oxyphil cells that were found contained numerous large mitochondria, some with whorls resembling those found in the chief cells. Only a few secretory granules were found in the cytoplasm of the oxyphil cells. No water-clear cells were observed. The cellular aggregates were composed of syncytial cells and small chief cells of similar appearance to those seen in normal dogs (3).

The light microscope observation of differing appearances of the follicle epithelium was confirmed. Its luminal surface exhibited microvilli and small bulbous projections. The lumina contained fluid, amorphous masses

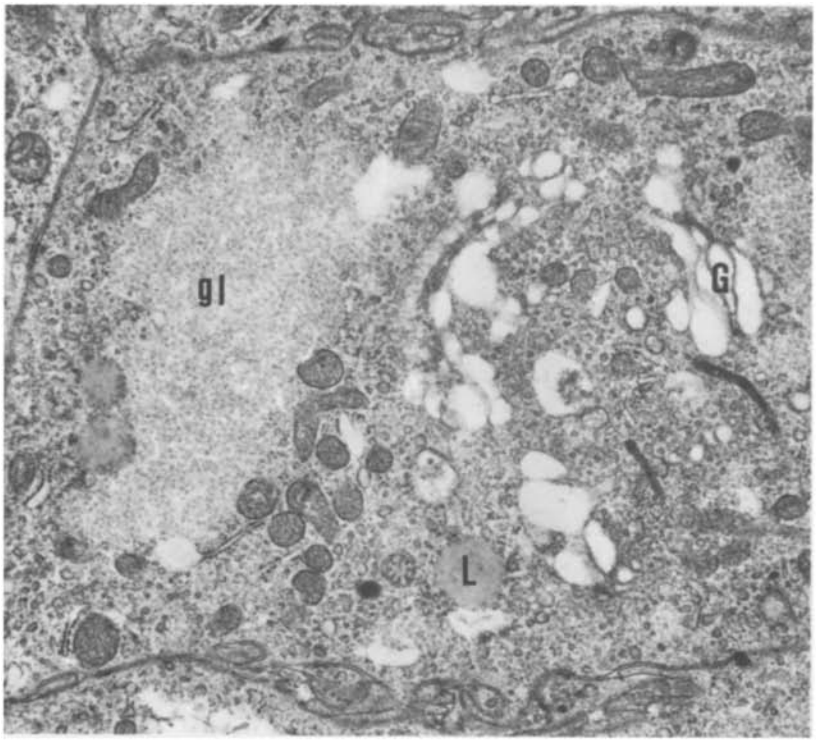

Fig. 5. Electron micrograph of parathyroid chief cell showing low electron density of cytoplasm, prominent Golgi complex (G), glycogen accumulation (gl), lipoid bodies (L), mitochondria, and cell membranes with occasional microvilli. X 20 000

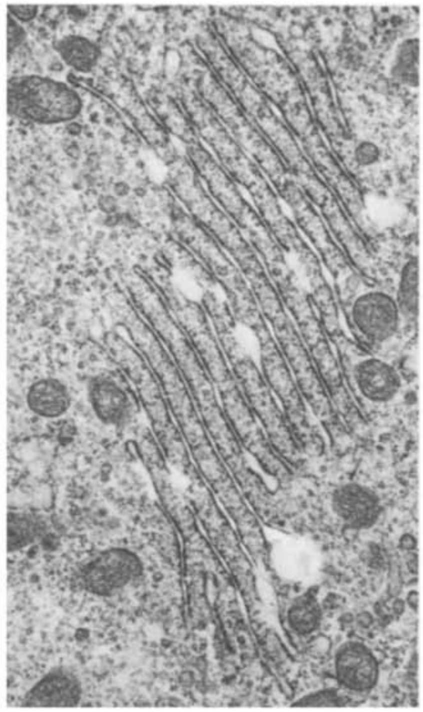

Fig. 6. Portion of chief cell cytoplasm showing prominent granular endoplasmic reticulum of lamellar type. X 24 000

Fig. 7. Chief cell cytoplasm containing smooth and rough-surfaced vacuoles that are either empty or contain amorphous or vesicular (v) particles. X 35 000

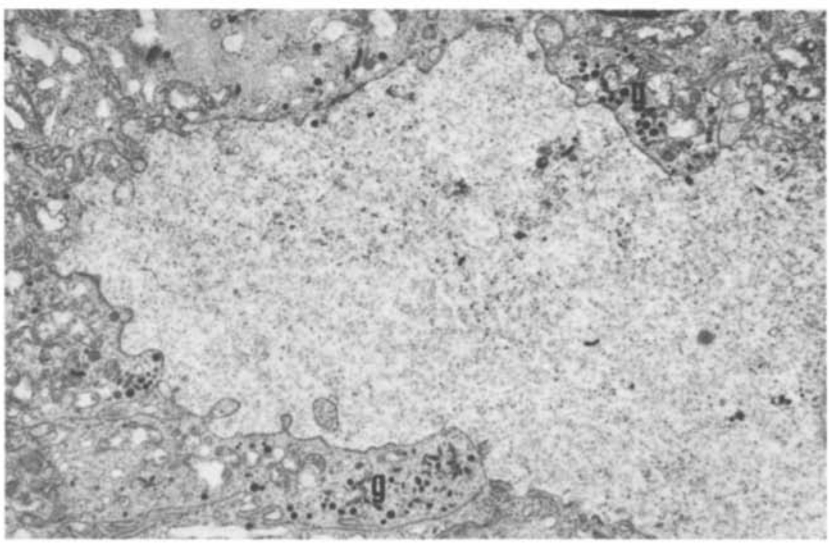

Fig. 8. Portion of parathyroid follicle lined by chief cells containing secretory granules (g) and occasional microvilli. Amorphous masses are seen in the lumen. X 8 500

(Fig. 8), desquamated epithelial cells, phagocytes, and cellular debris. Secretory granules could be seen in the apical portions of the surrounding parenchymal cells.

Discussion

The operations were effective in that moderate uraemia developed in all the dogs very shortly after the second operation. Light microscope examination at the end of the experimental period demonstrated hyperplasia of the remaining renal parenchyma, although it was not sufficient to relieve the laboratory features of renal failure.

The volumetric determinations and the results of light microscopy revealed parathyroid hyperplasia, and the fine structural changes indicated high activity of the parathyroid parenchymal cells. Nuclear size is related to functional activity: large nuclei occur in cells with high activity and small nuclei are seen in cells with low activity (cf. 11). The number of nuclear hits is also related to functional activity (12). In the present study there was an increased number of nuclear hits as compared with that in normal dogs, which suggests increased cellular activity. The microvilli, tortuosities, interdigitations of the cell mambranes (5), and the appearances of the follicles (6) may also be related to the altered functional state of the cells. Thus, both the quantitative and qualitative morphological alterations show that the animals had developed hyperparathyroidism in the presence of renal failure (secondary hyperparathyroidism).

The hyperplastic parathyroid glands were mainly composed of chief cells, which is in agreement with the findings of others (9, 23, 26, 31). Kretzschmar (22) has reported an increased number of waterclear cells in hyperplastic parathyroid glands from dogs, and, in dogs with chronic nephritis, fewer syncytial cells have been reported (9). The presence of whorls of cristae or tubules in the mitichondria of some parathyroid parenchymal cells may denote abnormal functioning of these organelles. Similar, but not identical, alterations have been seen in pancreatic B-cells in mice (4).

Parathormone (PTH) was not measured in these dogs. Using a biological method Highman and Hamilton (16) reported an increased level of PTH in secondary hyperparathyroidism in dogs. Slatopolsky et al. (35) found a twenty-fold increase of the PTH level in dogs with advanced experimental renal insufficiency.

Only total serum calcium was assayed in the present study. However, according to Wills and Lewin (38) there is a definite correlation in normal, hyper- and hypocalcaemic subjects, whether uraemic or not, between the total serum calcium level and the concentrations of the ultrafiltrable, ionized and protein-bound calcium fractions.

It is generally accepted that there may be normo- or hypocalcaemia in secondary hyperparathyroidism. Slatopolsky et al. (35) suggested that hypocalcaemia was a stimulus to the development of hyperparathyroidism in dogs, although hypocalcaemia was not observed in their animals. In rats with experimental renal disease the serum calcium level has been reported to be unchanged (21, 24); whereas marked hypercalcaemia was found 90 and 138 days after the development of experimental nephritis in rabbits (32).

The present study is the first in which the levels of serum calcium and serum phosphorus have been continuously followed in animals with experimental renal failure. The results show that secondary parathyroid hyperplasia and hyperfunction may develop in dogs in the presence of hypercalcaemia, and are consistent, therefore, with the findings in humans with secondary hyperparathyroidism (2).

The cause of secondary hyperparathyroidsm remains unknown, but it is believed that some factor or factors in the kidneys plays a role in the development of this type of parathyroid hyperfunction. In this connection it is of interest that, in recent years, specific inactivation of PTH by kidney tissue has been reported, and that failure of such inactivation has been suggested as a possible cause of secondary hyperparathyroidism (cf. 27). It has also been suggested recently that the kidney or its products may play an endocrinological role in normal calcium, phosphorus and bone metabolism (33). There is a definite need for further studies of the role of the kidney in calcium and phosphorus metabolism and its implications for the pathogenesis of secondary hyperparathyroidism.

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