Rat Hypoplastic Kidney (*hpk*/*hpk*) Induces Renal Anemia, Hyperparathyroidism, and Osteodystrophy at the End Stage of Renal Failure

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ABSTRACT. In rats with genetically hypoplastic kidneys (*hpk*/*hpk*) and associated hypogonadism (*hgn*/*hgn*), their kidneys contain only one-quarter the number of nephrons that are found in those of normal rats [26]. Not surprisingly, therefore, renal excretive function has been shown to be depressed in *hpk*/*hpk* rats [26]. In the study presented here, we have examined the process of the progression of renal failure and the development of renal secondary disease in *hpk*/*hpk* rats. The plasma concentrations of urea-nitrogen and creatinine were significantly higher in adult *hpk*/*hpk* rats than in normal rats. These values elevated gradually and the degree of renal histological damage also progressed with advancing age in the *hpk*/*hpk* rats. In addition, renal anemia appeared at 140 days of age or later in these rats, and hyperplasia of the parathyroid glands was visible macroscopically at 280 days of age. In the *hpk*/*hpk* rats plasma levels of calcium and phosphorus were significantly lower and higher than in normal rats, respectively, at 280 days of age. Pathologically, the left femora of *hpk*/*hpk* rats exhibited fibrous osteodystrophy at 280 days of age and the calcium content of the right femora (as a percentage of the dry weight of bone) was significantly lower than in normal rats at both 210 and 280 days of age. These results indicate that the reduced nephrogenesis of the *hpk*/*hpk* rats causes progressive renal failure, secondarily inducing anemia, hyperparathyroidism, and osteodystrophy.
— KEY WORDS: disease animal model, hyperparathyroidism, osteodystrophy, renal anemia, renal hypoplasia.

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Hypogonadic rats (*hgn*/*hgn*) exhibit male sterility and reduced female fertility as a single autosomal recessive trait [24, 25]. During a study of testicular pathogenesis, it was found that *hgn*/*hgn* rats were associated with bilateral hypoplastic kidneys (*hpk*/*hpk*) [27]. Thereafter, it was revealed that there is a smaller number (only one-quarter) of gromeluri in the kidneys of *hpk*/*hpk* rats than in normal ones, and that their plasma urea-nitrogen and creatinine concentrations were significantly higher than normal [26]. These results indicate that the renal function of *hpk*/*hpk* is depressed. We consider the *hpk*/*hpk* rats to be a good analytical tool with which to study the pathogenesis of renal hypoplasia and the mechanism of the progression from renal hypoplasia to renal failure. In general, the considerable loss of nephrons causes progressive chronic renal failure [1, 5, 14, 22] and, secondarily, induces renal anemia [3, 6, 12, 15, 29], hyperparathyroidism, and osteodystrophy at the end stage of renal failure [2, 6, 7, 8, 10, 11, 13, 16, 17, 20, 21, 23]. Although various animal models have been utilized in the study of these conditions [6, 12, 16, 17, 20, 21, 29], genetic animal models that could develop reproducibly chronic renal failure and induce renal secondary disease have been reported only rarely [11, 15]. In the report described here, in order to assess the possibility that *hpk*/*hpk* rats are a useful animal model in which we can study the process of progression from renal hypoplasia into renal failure, the process of the development of renal secondary disease, and the pharmacological effects of drugs designed to improve these conditions, we examined the clinical and pathological conditions of progressive renal failure in these rats during the period from adult to advanced age.

MATERIALS AND METHODS

Animals: The animals used in this study were male *hpk*/*hpk* rats and phenotypically normal litter mates (+/*hpk* or +/+) from the 24th generation of the HGN line maintained in our department [26]. The *hpk*/*hpk* males could be identified externally by their hypogonadism [26]. The rats were bred and fed under the same conditions as described in previous reports [24–27].

Hematological examination: Four groups of 4 *hpk*/*hpk* and 4 normal animals were used, one group at each of days 70, 140, 210, and 280 after birth. Blood samples were collected from the jugular vein with a plastic syringe moisturized with a small amount of heparin. Red and white blood cells were counted on a hemacytometer with the aid of a light microscope. The counts of reticulocytes and platelets were performed according to the Rees-Ecker method. The hematocrit was determined using a hematocrit tube and the centrifuging method. The concentration of hemoglobin was determined by the methemoglobin method. Plasma samples were obtained by centrifugation and stored at −40°C until they were used to obtain urea-nitrogen, creatinine, calcium, and phosphorus measurements.

Histological examination: After collecting blood samples from the rats they were sacrificed by ether overdose. Their kidneys and parathyroid glands with attached tracheas were removed and fixed in 4% neutral formalin, dehydrated in a graded alcohol series, embedded in paraffin, and then sectioned at 3 μm. The left femora of these rats were decalcified using 5% formic acid in formalin, and then embedded in paraffin. Renal sections were stained with

periodic acid and methenamine silver. The sections of other organs were stained with hematoxylin and eosin. The right femora were removed from the surrounding tissues and stored hermetically at −40°C until they were used to obtain calcium and phosphorus measurements.

Biochemical examination: Plasma urea-nitrogen, creatinine, calcium, and phosphorus levels were measured using the Fearon reaction (UreaN-Test, Wako), the Jaffe reaction (creatinine-Test, Wako), and the methylxylenol blue method (calciumE-Test, Wako), and p-methylphenol reduction method (phosphaC-Test, Wako), respectively. Following removal of the bone marrow, the stored right femora were crushed into small pieces. The pieces were kept at a temperature of 60°C until the dry weight of the bone was constant. They were then dissolved in a 1:1 (v/v) solution of nitric and perchloric acids. The solutions were evaporated on a heater which was kept at 150–250°C. The resulting powder was dissolved in 0.1N HCl. The solutions were diluted by 100 and 10 times to allow measurement of the calcium and phosphorus contents of the bone, respectively.

Statistical analysis: Student's *t*-test was used for the statistical analysis of the data, and the level of statistical significance was set at $p<0.05$ and $p<0.01$.

RESULTS

The urea-nitrogen and creatinine levels were significantly higher in adult *hpk*/*hpk* rats than in normal rats [26]. These values elevated gradually with age in the *hpk*/*hpk* rats (Fig. 1). Concomitant with these changes, renal pathological changes became severe in the *hpk*/*hpk* rats (Fig. 2). Dilatation of the lumen of the Bowman's capsules, thickening of the capsular walls, and infiltration of inflammatory cells into some portions of the interstitial tissues were observed in the *hpk*/*hpk* kidneys at 140 days after birth. At 280 days of age, glomerular sclerosis, cystic dilatation of the renal tubules, proteinaceous casts, and interstitial inflammation were observed in the *hpk*/*hpk* kidneys. Figure 3 shows the number of red blood cell counts, hematocrits, and the concentrations of hemoglobin in the *hpk*/*hpk* and normal rats at the days of age examined. These values were lower in the *hpk*/*hpk* than in the normal rats and there were significant differences in rats at 140 days and older. In spite of the occurrence of anemia, the number of reticurocytes did not increase and there was no significant difference between the *hpk*/*hpk* and normal rats in the number of both white blood cells and platelets (data not shown). Moreover, at 210 days of age, marked hyperplasia of the parathyroid glands was visible in 2 animals of 4 *hpk*/*hpk* rats. At 280 days of age, all of 4 *hpk*/*hpk* rats showed macroscopically visible hyperplasia of the parathyroid glands. Histologically, the parathyroid glands of the *hpk*/*hpk* rats exhibited hypertrophied chief cells with swollen nuclei (Fig. 4). Hypocalcemia and hyperphosphoremia were observed in the *hpk*/*hpk* rats, and the differences in these parameters between the *hpk*/*hpk* and normal rats were significant at 280 days of age (Fig. 5). Furthermore, the area of resorptive cavities increased and the bone marrow was replaced by osteoid and proliferating fibrous tissue in the femora of *hpk*/*hpk* rats at 280 days of age (Fig. 6). These pathological changes are similar to those referred to as fibrous osteodystrophy. The moderate pathological changes were found in the femora of *hpk*/*hpk* rats at 210 days of age (data not shown). The calcium content of the femora, as represented by the percentage of the dry weight of the bone, was significantly lower in the *hpk*/*hpk* compared to the normal rats at 210 and 280 days of age (Fig. 7).

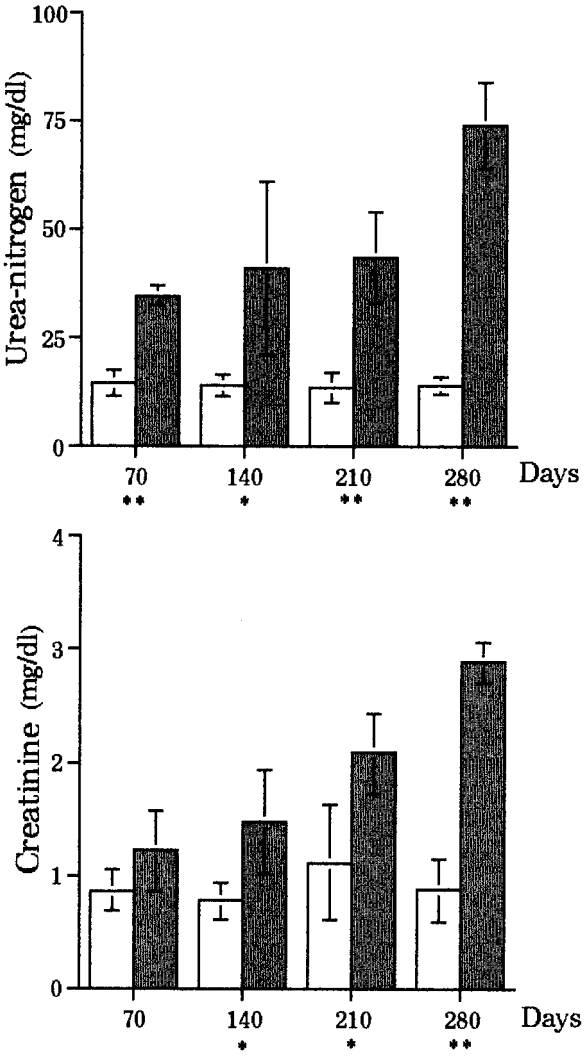

Fig. 1. Changes in plasma urea-nitrogen and creatinine levels of normal (□) and *hpk*/*hpk* (■) rats. Each data represents the average value with the standard deviation (SD). Significant differences in each parameter between *hpk*/*hpk* and normal rats are labeled as "*" ($p<0.05$) and "**" ($p<0.01$).

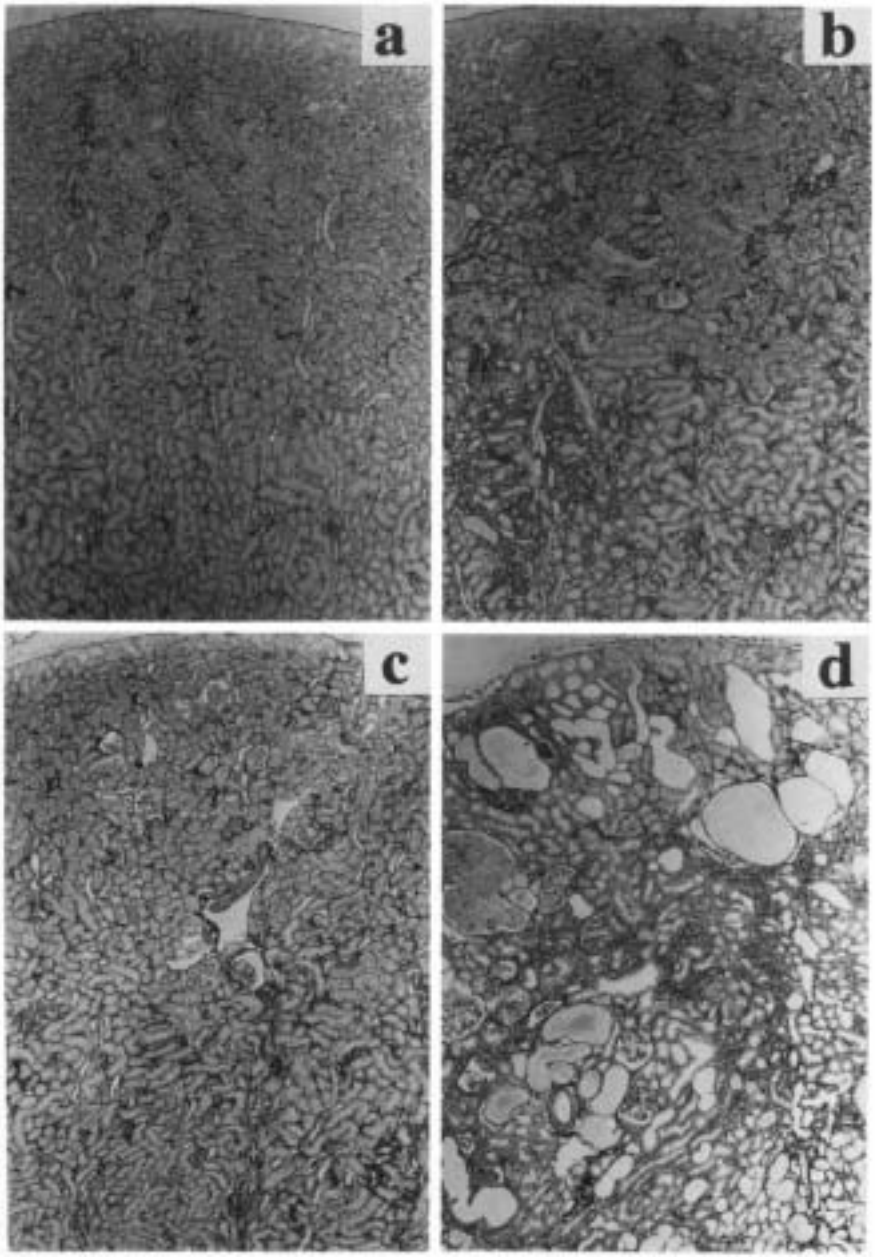

Fig. 2. Renal histology in normal (a, c) and *hpk*/*hpk* (b, d) rats at 140 (a, b) and 280 (c, d) days of age. The sizes of individual glomeruli are larger in the *hpk*/*hpk* (b) than in the normal kidney (a). Dilatation of the Bowman's capsular lumen, thickening of the pericapsular walls, and slight interstitial inflammation were observed in the *hpk*/*hpk* kidney at 140 days of age (b). More modest pathological changes, perhaps resulting from aging, were also observed at 280 days of age in the normal kidney (c). Glomerular sclerosis and cystic dilatation of the tubules with proteinaceous luminal casts were often observed at 280 days of age in the *hpk*/*hpk* kidney (d). The interstitial inflammation appeared to be more severe at 280 (d) than at 140 (b) days of age in the *hpk*/*hpk* kidney. Periodic acid and methenamine silver stain. × 30.

DISCUSSION

This report provides the hematological, biochemical, and pathological changes associated with progressive renal failure in *hpk*/*hpk* rats during the period from adult to advanced age. In our previous report, it was revealed that as a result of reduced nephrogenesis the number of glomeruli was significantly reduced in *hpk*/*hpk* compared to control rats [26]. It was also revealed that the individual nephrons were hypertrophied in the *hpk*/*hpk* kidneys, and that the

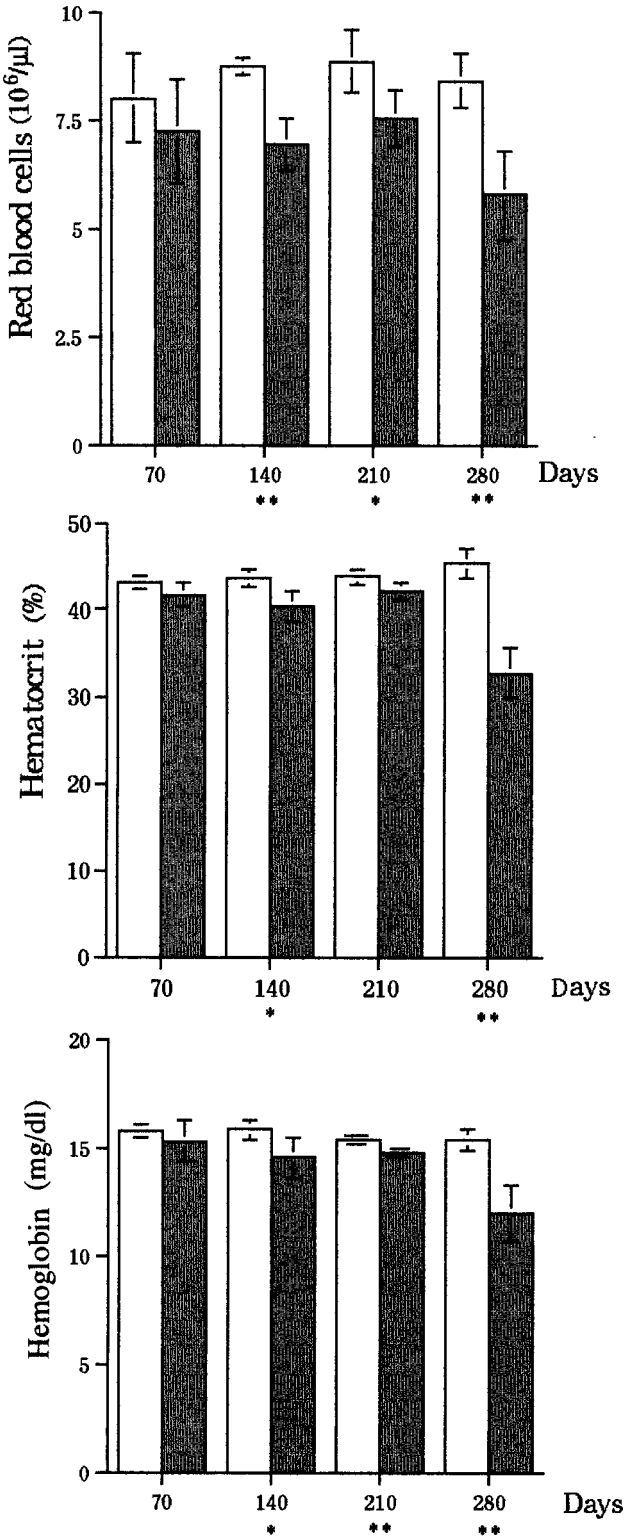

Fig. 3. Changes in the number of red blood cells, hematocrit, and concentration of hemoglobin of normal (□) and *hpk*/*hpk* (■) rats. Each data represents the average value with the SD. Significant differences in each parameter between *hpk*/*hpk* and normal rats are labeled as "*" ($p<0.05$) and "**" ($p<0.01$).

plasma urea-nitrogen and creatinine levels were significantly higher than normal [26]. The report described here has shown that *hpk*/*hpk* rats exhibit a progression of renal pathological changes, further increases in plasma urea-nitrogen and creatinine levels, renal anemia, hypocalcemia and hyperphosphoremia, hyperplasia of the parathyroid gland, and fibrous osteodystrophy in the femora at advanced age. The progression of chronic renal failure resulting from a genetic defect, a congenital reduced nephron mass, might be responsible for these renal secondary diseases in the *hpk*/*hpk* rats. It has been reported, in rat renal ablation models such as a five-sixth nephrectomy (80% reduction), that the glomerular hyperfiltration and hypertrophy that is induced by a considerable loss of nephrons causes glomerular sclerosis [1, 5]. These conditions will progress to chronic renal failure, secondarily renal anemia, hyperparathyroidism, and osteodystrophy [2, 6, 12–14, 16, 17, 22]. Some clinical case reports have indicated that renal hypoplasia progresses to chronic renal failure and causes renal secondary diseases at the end stage of renal failure [4, 23]. However, it has not been previously reported whether the genetically established animal model of congenital renal hypoplasia would induce renal failure and these secondary diseases. *bcl-2* deficient mice have been shown to exhibit renal hypoplasia during the embryonic stage [18]. However, these mice developed polycystic kidneys after birth and half of them had died by their 6th week of age [19]. The mouse *Os* gene is semidominant and lethal in homozygotes. It has been shown that the kidneys of *Os*/+ mice contain half of the number of nephrons present in the kidneys of +/+ mice [28]. Although molecular analytical evidence of glomerular sclerosis was obtained in the *Os*/+ mice, this mutant seemed not to progress to the end stage of renal failure [9]. The congenital three-quarters reduction in the number of nephrons in *hpk*/*hpk* rats could permit survival for a relatively long period of time, but will inevitably progress to chronic renal failure. This allows us to examine the process of the progression of renal failure and the development of secondary diseases in these rats.

The anemia observed in the *hpk*/*hpk* rats was aplastic and progressed with age. Therefore, the anemia is probably caused by the reduced production of hematogenic factors (e.g. erythropoietin) or a low responsiveness of the hematopoietic stem cells to these factors [3]. With regard to genetic animal models, the renal anemia in polycystic kidney disease has been reported to occur in DBA/2FG-pcy mice. The hemopoietic response to recombinant human erythropoietin has been examined in this mutant mouse [15]. Some studies have been carried out on erythropoietin, concerning its plasma concentration, production in the kidney, liver, and bone marrow, and the hemopoietic response in renal anemia [3, 6, 12, 15, 29]. The examination of a hemopoietic factor such as erythropoietin in *hpk*/*hpk* rats would produce useful information because of the congenital reduction in renal tissue, which is the major source of erythropoietin, that exists in these rats. The pathogenic mechanisms responsible for the development of

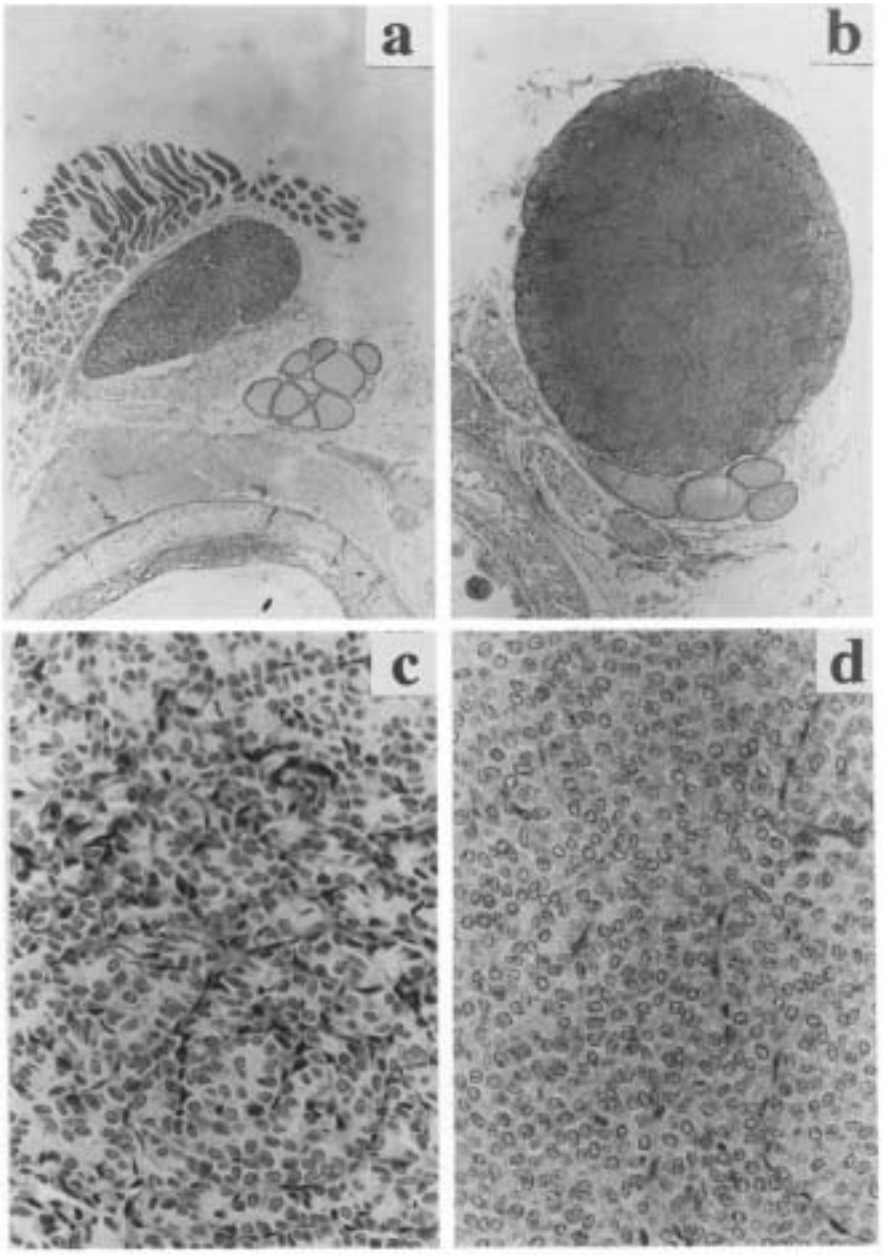

Fig. 4. Histology of the parathyroid gland in normal (a, c) and *hpk*/*hpk* (b, d) rats at 280 days of age. The size of the parathyroid gland is apparently larger in the *hpk*/*hpk* (b) than in the normal rat (a). The chief cells of the *hpk*/*hpk* parathyroid gland are hyperplastic and possess swollen nuclei, suggesting a hyperfunctioning condition (d). Hematoxylin and eosin stain. a, b: × 30, c, d: × 300.

renal hyperparathyroidism and osteodystrophy are not fully understood [8, 13, 20]. Hyperphosphoremia is considered to be caused by renal phosphate retention, resulting in calcium discharge. Renal damage potentially involves the reduced activation of vitamin D, inducing defective intestinal absorption of calcium. The resulting hypocalcemia is compensated for by the increased secretion of parathyroid hormone at the initial stage. If such a condition was continued for a long period, hyperparathyroidism would be prominent. Repetitive bone resorption will cause the development of pathological changes in bone [8, 13, 20]. The histopathological features of hyperplasia of the parathyroid glands and fibrous osteodystrophy of the femora in the *hpk*/*hpk* rats are similar to those observed in the rats with severe renal failure [7]. The spontaneous development of renal hyperplasia of the parathyroid glands and fibrous osteodystrophy have been reported to occur in aged rats suffering from glomerulonephrosis [2, 10] and in male rats

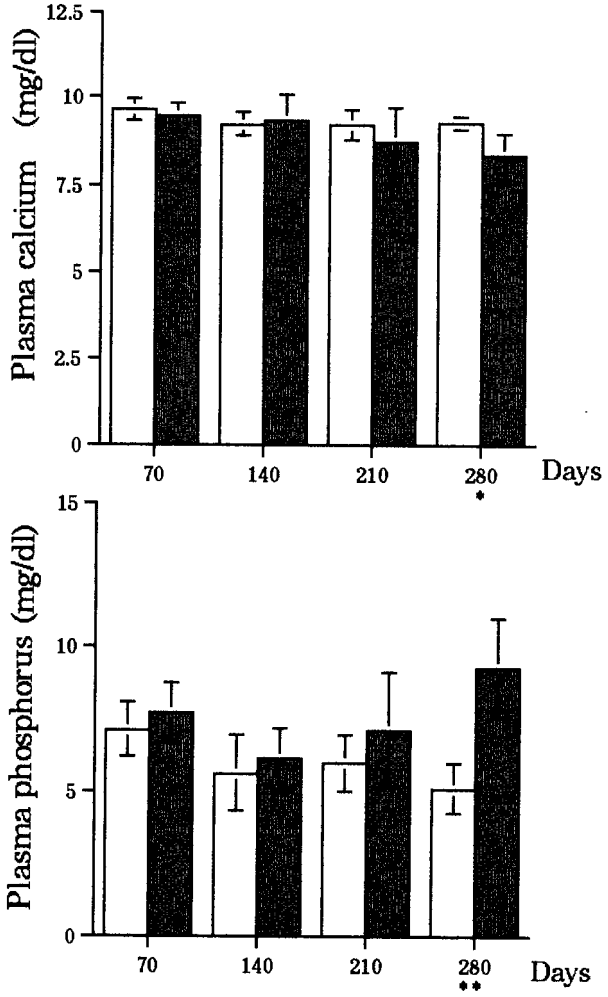

Fig. 5. Changes in plasma calcium and phosphorus levels of normal (□) and *hpk*/*hpk* (■) rats. Each data represents the average value with the SD. Significant differences in each parameter between *hpk*/*hpk* and normal rats are labeled as "*" ($p<0.05$) and "**" ($p<0.01$).

affected by the hereditary polycystic kidney disease inherited with an autosomal incomplete dominant trait [11]. With regard to animal models, this is the first report about these renal secondary diseases associated with hereditary renal hypoplasia.

Animal models of experimental renal failure have contributed to fundamental studies on the process of the progression of chronic renal failure and renal secondary diseases. Furthermore, these models have often been used in the practical examination of drugs that could inhibit the progression of these complications. In particular, experimental models involving mice and rats have provided useful information because their physiological and anatomical characterizations have been well established with regard to the kidneys and other organs. However, only a few reports exist in which the animal models exhibit induced renal secondary hyperparathyroidism and osteodystrophy. In remnant kidney models, a two-step nephrectomy or other treatments are necessary to induce chronic renal failure [6, 12, 16, 22]. In the experimental nephritis induced by the injection of sodium sulfacetylthiazole, the continuous administration of this drug is necessary to induce subchronic renal failure [21]. In the experimental progressive glomerulonephritis in rats reported by Sibata *et al*., renal osteodystrophy was induced by a single injection of a glycopeptide isolated from the renal cortical tissues of rats. In this model, however, renal damage varies between individuals [20]. In the models of renal failure induced by experimental treatments, nephrectomy, the administration of chemicals, and immunization are required and, we would have to consider the influence of the treatment itself on hematogenesis, the endocrine system, and metabolism. Since various factors are implicated in the development of renal secondary disease [8, 13, 22], models that possess both genetic uniformity and simplicity would be useful. The chronic renal failure that occurs in *hpk*/*hpk* rats results from the genetic reduction of nephrogenesis, causing the development of renal anemia, hypeparathyroidism, and osteodystrophy without the need for any experimental treatments. This suggests that *hpk*/*hpk* rats are a useful model in which we can study the process of the progression from renal hypoplasia to renal failure, the process of the development of renal secondary diseases, and the pharmacological effect of drugs designed to improve these conditions.

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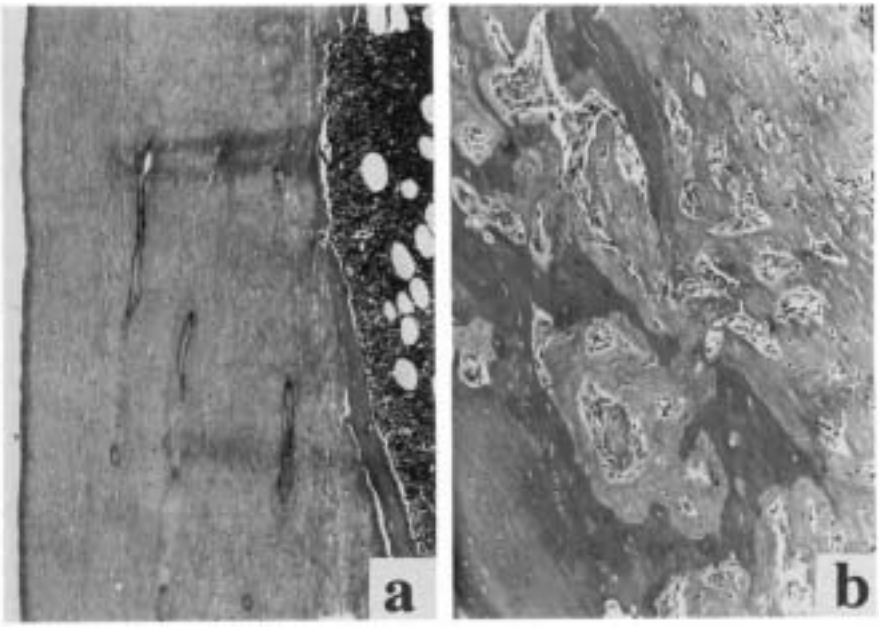

Fig. 6. Histology of the femoral diaphysis of normal (a) and *hpk*/*hpk* (b) rat at 280 days of age. The area of resorptive cavities was larger in the *hpk*/*hpk* (b) compared to the normal femora (a). The most comparable portions of the diaphysis between the *hpk*/*hpk* and normal rats are shown. The amount of bone marrow has decreased and the space in which the bone marrow would normally have existed has been replaced by osteoid bone containing fibrous tissue in the *hpk*/*hpk* rats (b). Hematoxylin and eosin stain. × 75.

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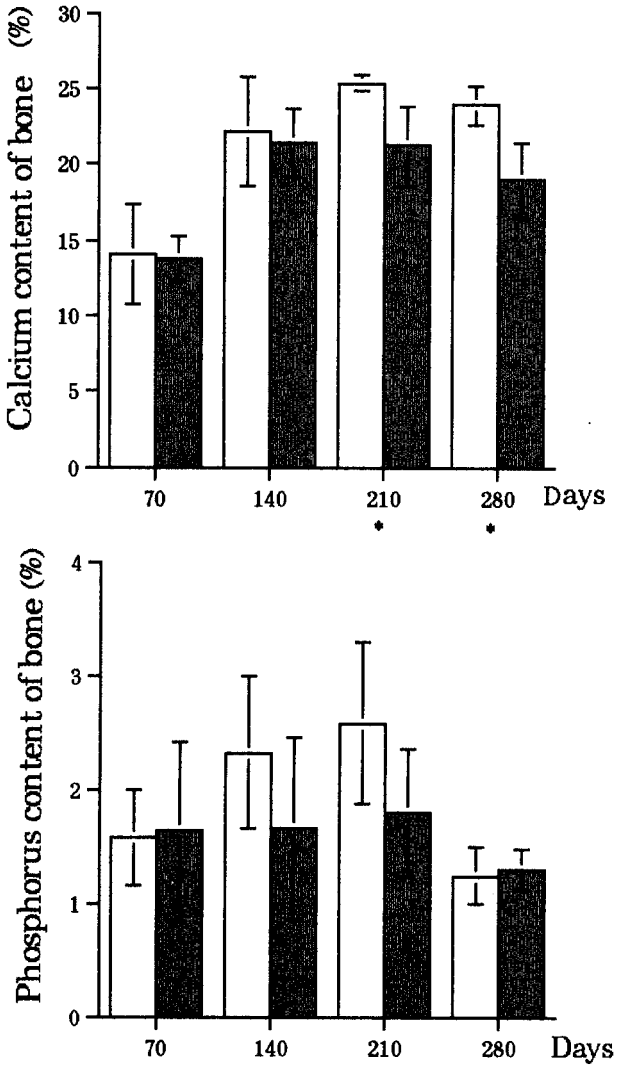

Fig. 7. Changes in the calcium and phosphorus content of the bone of normal (□) and *hpk*/*hpk* (■) rats, as represented by the percentage of the dry weight of the femoral bone. Each data represents the average value with the SD. Significant differences in each parameter between *hpk*/*hpk* and normal rats are labeled as "*" ($p<0.05$).

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