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Characterization of a Mouse Model of Chronic Uremia

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Summary. A mouse model of renal failure, which is induced by the sequential electrocoagulation of the right renal cortex and left nephrectomy, was examined for the capacity to reproduce the characteristics of chronic uremia. Assessment was conducted six weeks after the second surgical procedure in 13 week old female C57BL/6 inbred mice with renal failure and in normal and sham-operated controls. The surgery, which was well tolerated, was free of local and systemic signs of inflammation or infection. Growth was significantly delayed in all animals post surgery however renal failure mice presented the most severe growth retardation. Biochemical analysis of plasma revealed multiple abnormalities with commensurate elevations of urea and creatinine. In addition to the expected hyperphosphatemia, hyperkalemia and acidosis, a significant increase in cholesterol was present. Furthermore, in contrast to controls, renal failure mice produced large volumes of urine which contained significant levels of protein. Renal failure mice presented profound hematological changes in the red cell series in which anemia was evident. Changes in plasma biochemistry and in bone histology revealed the presence of severe secondary hyperparathyroidism. It was therefore concluded that the described mouse model of chronic renal failure presented characteristics consistent with those observed clinically in end-stage renal disease.

Key words: Experimental renal failure – Chronic renal failure – Chronic uremia

Introduction

The pathophysiology of experimental chronic renal failure remains to be clearly delineated. Animal studies using surgical models have received the most attention and most investigators have utilized the rat as the test species. Three basic models have been described in which renal lesions are created surgically with the purpose of inducing chronic renal failure without initiating concomitant infectious or inflammatory processes. The first is the remnant kidney model in which a subtotal nephrectomy is performed [2, 4]. The second model involves ligation of renal arteries [29]. Finally, papillectomy can be achieved successfully in some defined rat strains [2, 7].

Recently, we have described a model of chronic renal failure [10], in which mice are subjected to electrocoagulation of the surface of one kidney with subsequent contralateral nephrectomy according to a modification of a technique originally applied to rats [1]. The present model is of considerable practical as well as theoretical interest. The mouse is particularly well suited for immunological studies since its immunogenetic background and the function of its various immunocompetent cells and immunoglobulin classes have been extensively investigated. Accordingly, we initiated a study to examine the characteristics of this mouse model of chronic renal failure, particularly those related to extrarenal abnormalities secondary to uremia. Most studies were done approximately 6 weeks after the onset of renal failure, a time at which we had previously performed several immunological investigations [9, 12–16]. These experiments were conducted in female C57BL/6 inbred mice in which a number of biological features are well established [3, 23, 30]. The ability of chronic renal failure mice to thrive following nephrectomy was assessed by survival and growth rate. Routine biochemical and hematological studies were done to evaluate the systemic consequences of uremia and blood pressure determinations were conducted. Structural alteratons in bone were also assessed.

Methods

Animals

Experiments were performed in 5 week old female C57Bl/6 inbred mice (Canadian Breeding, St. Constant, Qué. and Kingston NY, USA)

left to acclimatize for one week in our animal facilities prior to use. All animals were fed a commercially available mouse diet (Ralston Purina Co., St. Louis, Mo, USA) containing approximately 20% protein by weight and provided in pellet form. Drinking water was untreated tap water. Food and water were available at libitum.

Production of Renal Failure

Renal failure was induced by a two-step procedure involving electrocoagulation of the surface of the surgically exposed right kidney and left nephrectomy. Details of this method have been reported previously [10]. Briefly, electrocoagulation of the entire surface of the right kidney except for a 2 mm of intact tissue around the hilum was followed by left nephrectomy twelve to fifteen days later. In sham-operated animals, the right kidney was electrocoagulated and the left kidney was temporarily exposed in a similar fashion to that used for nephrectomy but was not manipulated. All animals were subjected to electrocoagulation of the renal surface, nephrectomy or sham-surgery which were conducted under controlled ether anesthesia through small bilateral flank incisions leaving the intestines and the upper abdominal contents undisturbed. Renal electrocoagulation was performed using a foot-operated single point cauterizer angled at 30° (Hyfrecator, Model X-712, The Birtcher Corp., Los Angeles, Calif., USA). The kidney was freed from perirenal fat and the adrenal gland prior to electrocoagulation and special care was taken not to manipulate the ureter; after electrocoagulation, the kidney was replaced into the renal fossa and completely covered by the tissue of the abdominal wall and skin. After either surgical procedure, the incisions were closed in layers with clips applied to the skin. The duration of surgery from skin-to-skin never exceeded 10 min. Unless stated otherwise, the animals were studied 6 weeks after the second operation. The degree of renal failure was defined by the blood urea nitrogen (BUN) concentration as measured on sacrifice day.

Body Weight

Mice were weighed at the time of induction of renal failure, ear-clipped for future identification and weighed weekly until sacrifice.

Blood Pressure Measurement

Systemic blood pressure was measured in conscious mice by a tail-cuff method. For two weeks prior to assessment mice underwent daily acclimatization of the holding chambers required for blood pressure determinations. All measurements were made at the same time of day under standardized conditions.

Blood Tests

At the time of sacrifice blood was collected by cardiac puncture into plastic syringes coated with a 3:10 dilution of heparin (Hepalean, Harris Laboratories, Toronto, Canada). Blood urea nitrogen concentration was measured by autoanalyzer method either separately (IL9 Autoanalyzer, Instrumentation Laboratory Inc., Lexington, Mass., USA) or as part of SMAC-16 blood testing (Technicon Instruments Corporation, Montreal, Canada). Routine hematological assessment was done by Coulter Counter (Model 2B1, Coulter Diagnostics Inc., Hialeah, Fla., USA). In a separate experiment, platelet counts were also performed in parallel by direct phase-contrast microscopy [2]. Differential leucocyte counts were performed on the basis of 100 cells per slide on Wright-stained blood smears. In a limited number of mice reticulocyte counts were carried out on fresh and unstained thick blood smears.

Urine Biochemistry

Eighteen-hour urine collections were made in modified metabolic cages. Urinary protein content was measured with a Technicon RA 1000 Autoanalyzer (Technicon Instruments Corp., Montreal, Canada). Osmolarity was measured on frozen samples with an Advanced Cryomatic Osmometer (Model 3C2; Advanced Instruments Inc., Needham Heights, Mass., USA).

Bone Analysis

The bone was fixed in sucrose formalin buffer, then processed as previously described following embedding in glycol methacrylate, staining for acid phosphatase and counter-staining with modified Harris hematoxylin [21]. 2 μ undecalcified sections of the proximal tibia were examined.

Statistical Analysis

All results are expressed as means ± SD and single comparisons were made with Student's t test.

Results

Survival

Except for the occasional death occurring during surgery and ascribed to anesthesia, this model of chronic renal failure (mean BUN values of 100 mg/dl) was associated with a mortality rate which varied from 4 to 15% in separate experiments according to the degree of electrocoagulation applied to the right renal cortex. Deaths in renal failure mice occurred on the second to fourth day following left nephrectomy. Plasma biochemistry performed at that time in animals where death seemed imminent revealed values of BUN above 250 mg/dl and of potassium above 8 mmol/l. All animals that expired were excluded from analysis. In selected experiments where severe renal failure (BUN above 180 mg/dl) was induced by excessive cautery of the right kidney, increased mortality during the days following nephrectomy was observed (Fig. 1). Thereafter during the six week follow-up, no significant animal loss occurred. No post surgical fatalities were encountered in sham-operated control animals.

Growth

The effects of renal failure on growth were readily observed and growth curves of the three mice groups are presented in

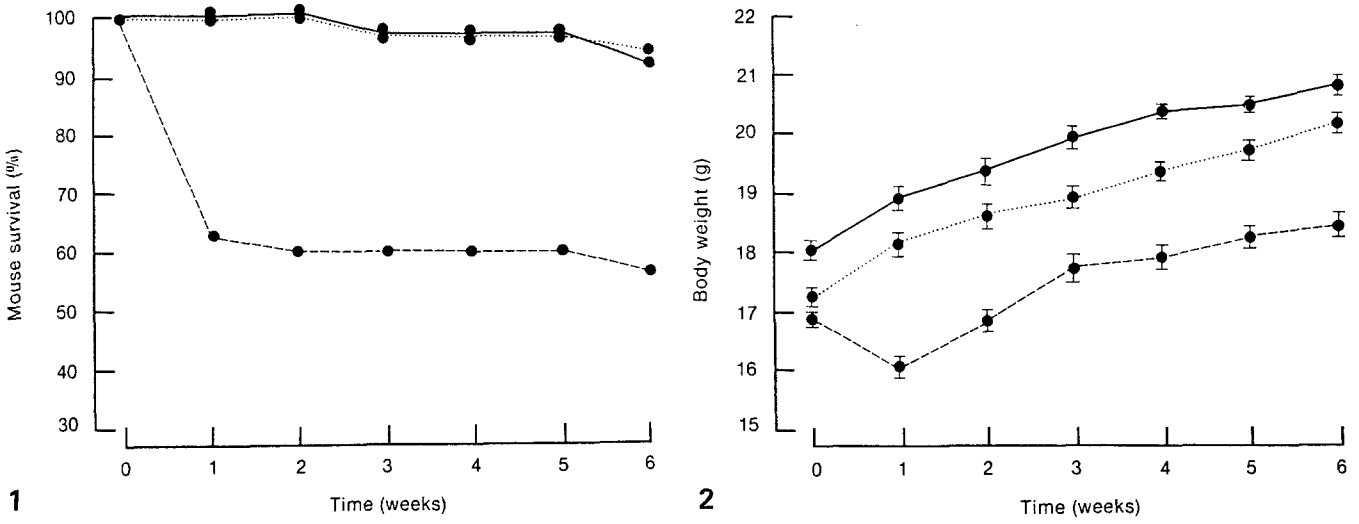


Fig 1. Survival curve of mice during the course of renal failure (*dashed line*, $n = 48$ at the start) and of sham-operated (*dotted line*, $n = 35$) and normal (*solid line*, $n = 36$) controls. Observations made in C57BL/6 female inbred mice during three separate experiments. All deaths past the first week were due to accidental overanesthesia

Fig 2. Growth curve in mice surviving during the course of renal failure (*dashed line*) and in sham-operated (*dotted line*) and normal (*solid line*) controls. Points and brackets represent the mean ± SD of at least 93 determinations obtained during the same experiments reported in Fig. 1

Fig. 2 where measurement began after the second surgical procedure. Following the second operation, there was a significant growth impairment in all animals. This impairment was most severe in renal failure mice in which significant weight loss was recorded one week after nephrectomy. Sham-operated animals recovered more quickly from the operation than renal failure mice and both groups had significant growth retardation at sacrifice six weeks later.

General Observations

In this study, none of the renal failure mice having survived the first week post nephrectomy demonstrated signs of spontaneous bleeding or gross neurological impairment. Furthermore, the nature and level of activity of renal failure mice as well as their overall appearance was comparable to control animals.

Local Findings

This surgical mode of induction of renal failure was free of local complications. The skin clips were removed one week after each of the two surgical procedures. On both occasions, in sham-operated and renal failure mice alike, the flank wounds were completely healed, and no signs of inflammation were detectable. Examination of the peritoneal cavity six weeks after the second surgery revealed no abnormalities outside of the kidneys. Again, there were no signs of inflammation or infection. The intraperitoneal side of the surgical wounds had healed completely. The general appearance of the electrocoagulated kidney of renal failure mice was markedly altered. The outer surface was pale, irregular and distended with translucent areas through which urine could be seen. When punctured the kidney rapidly emptied of urine and collapsed to a thin-walled pouch. In contrast, the electrocoagulated kidney of sham-operated animals had markedly atrophied and was generally difficult to locate, appearing as a very small mass of nondescript tissue adhering firmly to the liver. In these animals marked hypertrophy of the contralateral kidney was always present.

Biochemical Evaluation

The biochemical data of mice with renal failure and sham-operated and normal controls are shown in Table 1. A number of species differences between mice and man can be appreciated, the most striking involving creatinine, bicarbonate, phosphate, cholesterol, and the enzymes GOT, GGTR and LDH. The expected retention of nitrogenous compounds was observed in renal failure mice. Commensurate increases of urea and creatinine occurred averaging fivefold and two to threefold, respectively. In sham-operated animals, values of urea and creatinine did not change significantly, indicative of the degree of contralateral renal hypertrophy that followed the extensive destruction of the right kidney by electrocoagulation. In contrast to control animals, renal failure mice presented moderately, although significantly, elevated mean values of plasma potassium, chloride, calcium and phosphate as well as significantly reduced bicarbonate concentrations. Furthermore, renal failure mice always presented biochemical evidence of

Table 1. Plasma concentrations of selected blood constituents in mice six weeks after the onset of renal failure and in sham-operated and normal controls[a]

Status of animals	Normal $n = 32$	Sham-operated $n = 32$	Renal failure $n = 30$	Normal range in man
Glucose (mg/dl)	223 ± 31	218 ± 48	198 ± 26	60 –115
Urea nitrogen (mg/dl)	19 ± 3	24 ± 5	105 ± 36[c,d]	8 – 25
Creatinine (mg/dl)	0.4 ± 0.1	0.4 ± 0.1	0.9 ± 0.3[c,d]	0.5– 1.5
Sodium (mmol/l)	147 ± 6	140 ± 13	148 ± 6	136 –147
Potassium (mmol/l)	4.2 ± 0.7	4.4 ± 0.9	5.5 ± 0.9[c,d]	3.5– 5.0
Bicarbonate (mmol/l)	12 ± 2	11 ± 3	9 ± 3[c,d]	21 – 30
Chloride (mmol/l)	109 ± 4	102 ± 8[e]	119 ± 6[c,d]	97 –109
Uric acid (mg/dl)	3.0 ± 0.6	3.6 ± 1.2[e]	2.6 ± 1.2[c,d]	2.5– 8.5
Calcium (mg/dl)	9.3 ± 0.7	9.8 ± 0.9[e]	10.6 ± 1.0[c,d]	9.0– 10.6
Phosphate (mg/dl)	7.4 ± 1.0	6.6 ± 0.9[e]	8.8 ± 1.6[c,d]	2.4– 4.5
Total protein (g/dl)	5.2 ± 0.4	5.1 ± 0.7	5.2 ± 0.3	6.0– 8.0
Albumin (g/dl)	3.0 ± 0.3	3.0 ± 0.4	3.0 ± 0.3	3.5– 5.2
Cholesterol (mg/dl)	110 ± 16	119 ± 21	188 ± 40[c,d]	110 –250
Bilirubin (mg/dl)	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2– 1.2
Alkaline phosphatase (U/l)	112 ± 25	124 ± 20[e]	307 ± 106[c,d]	30 –110
GOT(U/l)[b]	221 ± 125	201 ± 85	226 ± 207	8 – 40
GPT (U/l)[b]	58 ± 51	35 ± 24[e]	30 ± 28[c]	0 – 40
GGTR (U/l)[b]	3 ± 2	3 ± 3	2 ± 1[c]	7 – 55
LDH (U/l)[b]	393 ± 198	382 ± 134	366 ± 181	100 –216
Globulins (g/dl)	2.2 ± 0.4	2.1 ± 0.6	2.2 ± 0.3	2.3– 3.3

[a] Determinations performed at sacrifice in 13 week old female inbred C57BL/6 mice and expressed as mean ± SD
[b] Abbreviations used are: *GOT* = glutamic oxaloacetic transaminase; *GPT* = glutamic pyruvic transaminase; *GGTR* = gamma glutamyl transferase; *LDH* = lactate dehydrogenase
[c–d] Indicates a significant difference ($p < 0.05$) between renal failure mice and [c] normal mice and [d] sham-operated mice
[e] Indicates a significant difference ($p < 0.05$) between sham-operated and normal mice

Table 2. Urine biochemistry in mice six weeks after the onset of renal failure and in sham-operated and normal controls[a]

Status of animals	Normal $n = 14$	Sham-operated $n = 14$	Renal failure $n = 11$
BUN (mg/dl)	26.1 ± 5.0	25.3 ± 3.5	119.3 ± 21.6[b,c]
Volume (ml)	1.5 ± 0.8	2.2 ± 0.8	3.0 ± 1.3[b]
Osmolarity (mOsm/kg H_2O)	417.6 ± 163.9	307.5 ± 86.7	338.5 ± 107.0
Protein (mg/ml)	0.12 ± 0.05	0.13 ± 0.05	0.47 ± 0.4[b,c]

[a] Eighteen hour urine collection performed the day before sacrifice in 13 week old female inbred C57BL/6 mice; values given are means ± SD
[b–c] Indicates a significant difference ($p < 0.05$) between renal failure mice and [b] normal mice and [c] sham-operated mice

secondary hyperparathyroidism with hyperphosphatemia and elevated alkaline phosphatase in the absence of hypocalcemia. An unexpected finding in this study was the presence of hypophosphatemia in the sham-operated animals. Interestingly, renal failure mice presented significantly elevated levels of plasma cholesterol compared to controls. The other biochemical parameters tested were similar across the three groups of mice.

Urinary characteristics of specimens collected from mice during an 18 hour collection period are presented in Table 2. Renal failure mice were not oliguric and in fact produced the greatest volumes of urine and sham-operated mice also produced increased urine volumes compared to normal controls. Significant levels of protein were recovered from urine specimens collected from renal failure mice while urine osmolarity was not significantly different between renal failure and control animals.

Hematological Assessment

After six weeks of renal failure, there was a marked decrease in hemoglobin concentration (Table 3) as well as erythrocyte number and hematocrit (data not shown). A close relationship was observed between the fall in hemoglobin and the severity of the renal failure (Fig. 3). Erythrocyte indices were only moderately, although significantly, affected by renal failure where mean corpuscular volume (MCV) and

Table 3. Hematological features of mice six weeks after the onset of renal failure and in sham-operated and normal controls[a]

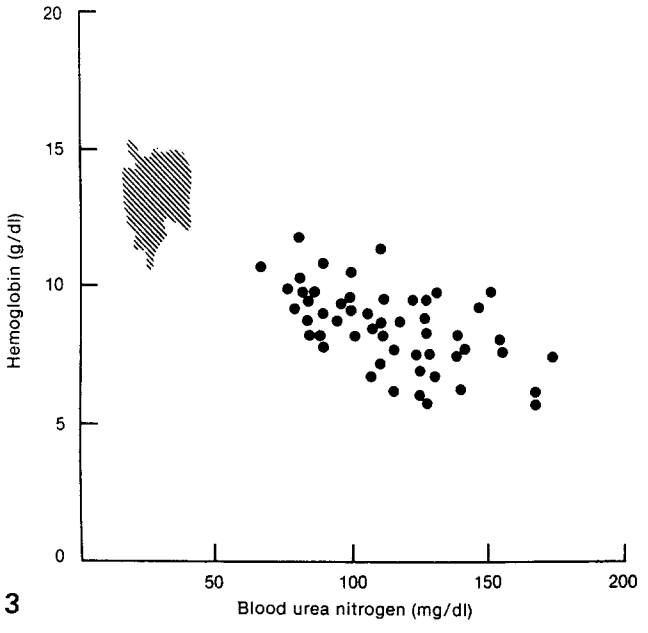
Status of animals	Normal $n = 68$	Sham-operated $n = 64$	Renal failure $n = 56$	Normal range in man
BUN (mg/dl)[b]	25 ± 6	29 ± 6	108 ± 33[d,e]	8– 25
Leucocytes/mm^3	5,334 ± 2,937	6,125 ± 2,269	5,188 ± 3,278	4,800–10,800
Neutrophils (%)	5.3 ± 3.6	6.1 ± 3.6	7.3 ± 5.8[d]	–
Neutrophils/mm^3	295 ± 328	379 ± 277	341 ± 272	–
Lymphocytes (%)	94.3 ± 4.0	93.5 ± 3.6	92.3 ± 6.0[d]	–
Lymphocytes/mm^3	4,910 ± 2,723	5,725 ± 2,144	4,891 ± 3,144	–
Platelets (x 10^3/mm^3)	813 ± 222	804 ± 193	807 ± 203	130– 400
Hemoglobin (g/dl)	13.4 ± 0.9	13.3 ± 0.8	8.4 ± 1.4[d,e]	14– 18
MCV (μm^3)[b]	45.7 ± 1.0	45.4 ± 1.1	44.1 ± 1.0[d,e]	82– 100
MCH (pg)[b]	16.4 ± 0.7	16.5 ± 0.7	15.6 ± 0.6[d,e]	27– 31
MCHC (g/dl)[b]	35.8 ± 1.4	36.4 ± 1.9	35.3 ± 1.6[e]	32– 36
Reticulocytes (%)[c]	2.5 ± 0.9	2.7 ± 0.6	1.8 ± 0.7[e]	–

[a] Determinations performed at sacrifice in 13 week old female inbred C57BL/6 mice and expressed as mean ± SD

[b] Abbreviations used are: BUN, blood urea nitrogen; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration

[c] Reticulocyte counts were performed in only eight animals of each group and are not corrected for the degree of anemia

[d–e] Indicates a significant difference ($p < 0.05$) between renal failure and [d] normal mice and [e] sham-operated mice



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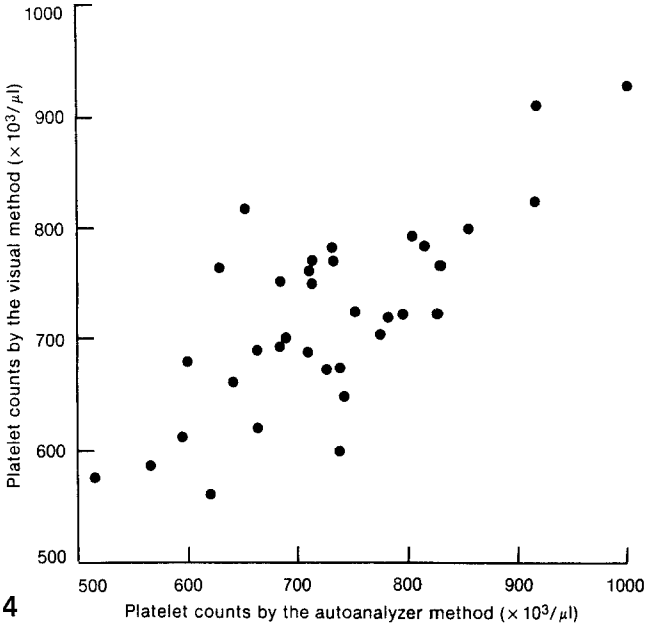

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Fig 3. Correlation between concentrations of blood urea nitrogen and hemoglobin ($y = -0.03x + 12.07$, $r = -0.608$, $p < 0.01$) of 53 mice 6 weeks after the onset of renal failure. Values for sham-operated and normal controls are contained within the shaded area. Other hematological features of the mice are presented in Table 3

Fig. 4. Correlation between circulating platelet numbers of 35 normal 6 week old C57BL/6 female inbred mice as determined by the visual and autoanalyzer methods. ($y = 72x + 90$, $r = 0.852$, $p < 0.01$)

mean corpuscular hemoglobin (MCH) were reduced compared to control mice. It is noteworthy that normal values of MCV and MCH in the mouse are approximately half that observed in man. Reticulocyte counts indicative of the extent of bone marrow response to the anemia tended to be reduced in renal failure mice and when corrected for the degree of anemia reticulocytosis was even more indicative of an inadequate bone marrow response.

Examination of the total and differential leucocyte counts and platelet numbers, revealed no differences between renal failure mice and controls (Table 3). Circulating leucocytes in the mouse are predominantly lymphocytes and polymorphs constitute a small minority, approximately 5%, of circulating leucocytes while monocytes are rarely seen [9]. Platelet numbers are consistently higher than in man. The standard method to ascertain platelet counts was to use the autoanalyzer counter. However, the counter is set for human blood in which erythrocytes are considerably larger than platelets but in the mouse erythrocytes are small and in fact close to the size of platelets. Therefore

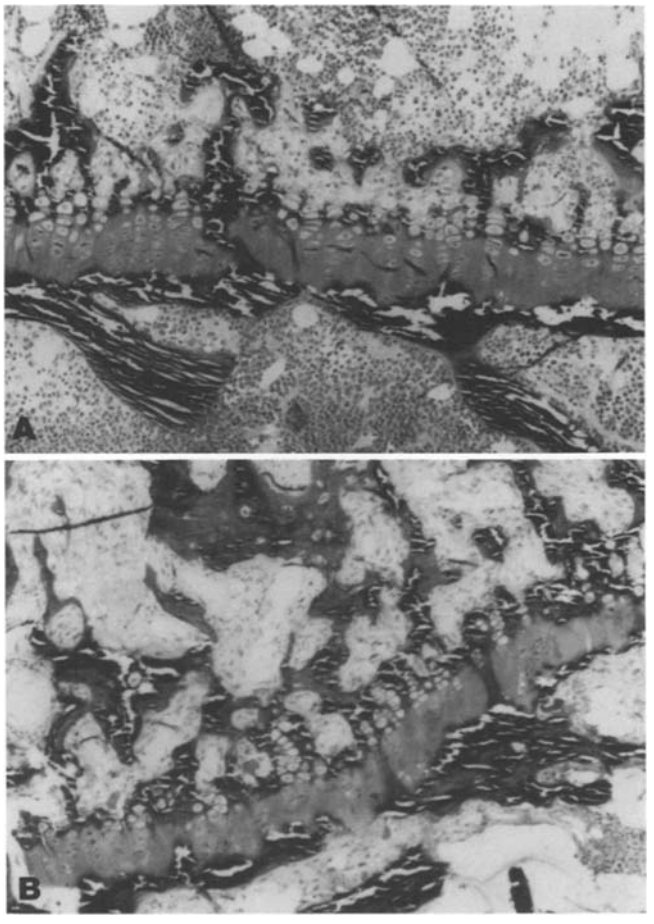

Fig. 5A and B. Examples of bone sections (upper metaphyseal plate of tibia) in normal mice (**A**) and in mice six weeks after the onset of renal failure (**B**). The prominence of osteoclasts, bone remodeling and marrow fibrosis is visible during renal failure (Acid phosphatase stain with Harris hematoxylin counterstain; original magnification x 170) (Courtesy of Dr. Michael Kaye)

the validity of mouse platelet counts based on assessment by the autoanalyzer method was confirmed by conventional counting of platelets employing visual means (Fig. 4).

Bone Studies

Renal osteodystrophy was fully established 6 weeks after the onset of renal failure. Changes in bone histology consistent with severe hyperparathyroidism were observed with an increase in the number of osteoclasts, extensive areas of increased bone resorption and widespread marrow fibrosis. Differences in bone histology between normal and renal failure mice can be appreciated in Fig. 5.

Blood Pressure

Systolic blood pressure determinations (tail cuff technique) were conducted in a small number (4 animals in each group) of renal failure mice (112 ± 13 mmHg) (mean ± SD) and their sham-operated (94 ± 9) and normal (103 ± 4) controls. No significant differences in systolic blood pressure between the three animal groups were recorded.

Discussion

This report describes abnormalities in mice with chronic renal failure which resemble those observed in humans with end-stage renal disease. Significant growth retardation, severe anemia, major alterations in blood chemistry and striking secondary hyperparathyroidism were observed in female C57BL/6 inbred mice after six weeks of renal failure. The same abnormalities are found in humans and it was concluded that this mouse preparation is a suitable animal model of chronic uremia. The results of this study confirm our earlier findings [9, 12–16] in that a significant reduction in renal function is consistently achieved with the ensuing retention of nitrogenous products and severe consequences of the renal failure readily follow. Previous reports [8, 17, 27, 28] supports these data, indicating a substantial increase in blood urea nitrogen for prolonged periods in similar experimental mouse models following controlled injury to both kidneys. In the present model we have previously shown that the severity of the renal failure is greatest if the electrocoagulation is limited to one kidney with a subsequent contralateral nephrectomy [10].

It was our goal to develop a surgical preparation with a consistent and reproducible degree of renal failure which could be readily induced in a large number of mice that could then be maintained under standard conditions of animal husbandry. After an initial animal loss during the first week post nephrectomy due to excessive renal failure in a small proportion of mice, no significant mortality was observed during a six week follow-up period. According to previous observations six weeks after the second surgical procedure, renal failure and sham-operated mice did not present evidence of infection or inflammation consequent to the surgical preparation [15, 16]. Peritoneal leucocytes collected from renal failure mice were similar to a resident population and peritoneal structures were sterile as evidenced by microbiological assessment.

This mouse preparation of renal failure was originally developed for the study of immune responses in chronic experimental uremia. In that context, sham-operated animals were subjected to electrocoagulation of the right kidney at the time of the first surgery and mobilization rather than removal of the contralateral kidney two weeks later. Therefore the potential immunomodulating influence of thermal injury to the kidney as well as the surgical trauma inherent to the model was accounted for in the sham-operated control animal [18, 20]. The present study demonstrated that the level of nitrogenous waste products of sham-operated and normal mice were not significantly different.

Postoperatively, growth of uremic mice was significantly impaired and six weeks after the induction of renal failure mean body weights had decreased to 80% of normal mice. Although growth rates were accelerated 2 and 3 weeks after surgery, renal failure mice were smaller and actually gained less weight than did sham-operated controls. In previous experiments we have shown that sham-operated mice demonstrated catch-up growth whereas renal failure animals continued to present growth retardation up to 15 weeks after the induction of renal failure [11].

The remarkable difference in the appearance of the electrocoagulated kidney between renal failure mice and sham-operated controls might find an explanation in the phenomenon of renal counterbalance [6]. It has been known for a long time that the effect of unilateral renal damage on the function of the affected kidney depends on the function of the contralateral kidney. The exact mechanism of renal counterbalance is less clear. Recent studies suggest that this occurs as a result of either increases in vasodilatory substances or decreases in vascoconstrictor compounds. Alternately this response may be mediated by the accumulation of naturally occurring cytoprotective agents. Evidence for the physiological importance of renal counterbalance was brought about by a number of experimental observations, particularly by unilateral ureteral ligation or renal artery clamping in the rat. Instead, the electrocoagulation technique that we used in our study produces an inhomogenous renal injury leaving a rim of renal cortex around the hilum intact and the deep juxtamedullary nephrons which may have escaped the thermal injury.

When compared to adult man, we found that differences in normal blood constituents were common in thirteen week old female C57BL/6 inbred mice. These differences included an increase in inorganic phosphate, the enzymes alkaline phosphatase, GOT and LDH, and in the number of platelets and circulating lymphocytes. As well a decrease in creatinine, bicarbonate, cholesterol, bilirubin, the enzyme GGTR, the erythrocyte indices MCV and MCH and in polymorphonuclear counts was demonstrated. Elevations in blood glucose could have resulted from the scheduling of our evaluation in the early morning in mice with nocturnal eating habits. The sodium-containing heparin preparation used for the blood collection might explain the observed hypernatremia relative to man.

Normal blood levels of creatinine in mice comparable to that of man have been observed by several groups [3, 23, 30]. Meyer et al. [25] have demonstrated the overestimation of the picric acid method in measuring serum creatinine levels in mice. In studies of normal C57BL/6 female emice, we have observed a similar fourfold difference between two automated methods of determination of plasma creatinine of normal mice, using the SMAC equipment in the present report and the 1L-1 elsewhere [15]. One difficulty with the former method is that the volume required by that technique is large for mice. In addition, even the latter method is still insensitive to very low levels of creatinine. For these reasons the measurement of blood urea nitrogen constituted our routine index of evaluation of renal function in mice.

Biochemistry assessments of renal failure and control mice revealed several interesting manifestations of uremia. Renal failure mice presented evidence of secondary hyperparathyroidism, with elevated plasma levels of inorganic phosphate and alkaline phsophatase. Hypocalcemia, described as a complication of chronic renal failure and attributed to the rise of serum phosphate and vitamin D deficiency was not observed in these chronically uremic mice. Although we did not measure ionized calcium in the present study, it is very likely that increments in total serum calcium in chronically uremic mice reflect a rise in ionized calcium. It is currently not feasible to perform measurement of parathyroid hormone in the mouse and therefore no conclusions regarding such measurements can be drawn from the current data.

In contrast to the evident secondary hyperparathyroidism observed in the chronically uremic mice, investigations of sham-operated mice unexpectedly revealed hypophosphatemia, hypercalcemia and high alkaline phosphatase. These biochemical findings are characteristic of primary hyperparathyroidism yet sham-operated animals had undergone renal electrocoagulation yielding a reduction of renal parenchyma. Therefore the observed hyperparathyroidism must in fact be of a secondary nature. We do not have a satisfactory explanation for the signs of early secondary hyperparathyroidism observed in the sham-operated mice.

Much like in man, the hematological consequences of chronic renal failure in mice affected predominantly the red cell series [5]. Six weeks after the onset of renal failure mice invariably presented severe anemia with evidence of relative bone marrow unresponsiveness. We have demonstrated previously the progressive development of the anemia from the time of onset of the renal failure [11]. In view of the prominence of the bone disease the anemia could result in part from excess parathyroid hormone through at least three pathways [24]. These include inhibition of erythropoiesis, shortening erythrocyte survival and inducing fibrosis of the bone marrow cavity. Thus the overall effects of the multiple consequences of uremia on hematopoiesis may result in prevailing functional alterations which may also be accompanied by structural changes. The observations made in this study would suggest that bone marrow fibrosis might play an important contributing role in the anemia of severe renal failure.

Our results confirm and extend the observations of others who have developed animal models of experimental uremia through surgical reduction of renal parenchyma and have assessed renal failure induced systemic changes. However, our model is singular in that it provides the means for studying the role of renal failure in modulating immune responses. The availability of immunodeficient and immunomodified strains, and the volume of knowledge on immune cell populations and membrane markers favor the mouse in studies of the potential influence of renal failure on immune function.

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References

1. Boudet J, Man NK, Pils P, Sausse A, Funck Bretano JL (1978) Experimental chronic renal failure in the rat by electrocoagulation of the renal cortex. Kidney Int 14:82–86
2. Brecher G, Cronkite EP (1950) Morphology and enumeration of human blood platelets. J Appl Physiol 3:365–377
3. Crispens CG Jr (1975) Handbook on the laboratory mouse. Charles C. Thomas, Springfield, Illinois
4. Da Costa Silva A, Albuquerque ZP (1978) Experimental model of chronic renal failure in rats. Nephron 20:297–298
5. Eschbach JW, Adamson JW (1985) Anemia of end-stage renal disease. Kidney Int 28:1–5
6. Finn WF (1985) Renal counterbalance. J Lab Clin Med 105: 523–530
7. Finkelstein FO, Hayslett JP (1974) Role of medullary structures in the functional adaptation of renal insufficiency. Kidney Int 6:419–425
8. Gabizon D, Goren E, Shake DU, Averbukh Z, Rosenmann E, Modai D (1985) Induction of chronic renal failure in the mouse: a new model. Nephron 40:349–352
9. Gagnon RF (1986) Delayed-type hypersensitivity skin reaction in the chronically uremic mouse: influence of severity and duration of uremia on the development of response. Nephron 43:16–21
10. Gagnon RF, Duguid WP (1983) A reproducible model for chronic renal failure in the mouse. Urol Res 11:11–14
11. Gagnon RF, Ansari M (in press) Development and progression of uremic changes in the mouse with surgically induced renal failure. (in press)
12. Gagnon RF, Lu DSK (1985) Mechanism of depressed immunity in chronic renal failure; effect of cyclophosphamide pretreatment on delayed-type hypersensitivity skin reaction. J Clin Lab Immunol 18:135–140
13. Gagnon RF, Gold J, Gerstein W (1984/85) A mouse model for delayed-type hypersensitivity skin changes in chronic renal failure. Uremia Invest 8:121–125
14. Gallimore B, Gagnon RF, Richards GK (1986) Intraperitoneal challenge with *Staphylococcus epidermidis* in chronically uremic mice: effect of inoculum size. In: Khanna R, Nolph KD, Prowant B, Twardowski ZJ, Oreopoulos DG (eds) Advances in continuous ambulatory peritoneal dialysis. University of Toronto Press, pp 121–124
15. Gallimore B, Gagnon RF, Stevenson MM (1986) Cytotoxicity of commercial peritoneal dialysis solutions towards peritoneal cells of chronically uremic mice. Nephron 43:283–289
16. Gallimore B, Gagnon RF, Richards GK (1987) Response to intraperitoneal *Staphylococcus epidermidis* challenge in renal failure mice. Kidney Int 32:678–683
17. Gibb IA (1985) An experimental model of chronic renal failure in mice. Clin Immunol Immunopathol 35:276–284
18. Grouls V, Helpap B (1978) DNA-synthesis in the rat thymus after focal thermolesions on abdominal organs. Res Exp Med 173:229–237
19. Harley J (1967) Hematology of rats and mice. In: Cotchin E, Roe FJC (eds) Pathology of laboratory rats and mice. Blackwell, Oxford, pp 501–536
20. Helpap B, Kaiser R (1981) The cellular response of T and B dependent areas of the spleen after focal thermocoagulation of liver and kidney. Virchows Arch 36:291–302
21. Kaye M, Zucker SW, Leclerc YG, Prichard S, Hodsman AB, Barré P-E (1985) Osteoclast enlargement in endstage renal disease. Kidney Int 27:574–581
22. Kroon DB, Jongkind JF, Wisse JH (1962) Resection of the renal papillae in rats. Experentia 18:581–583
23. Jackson Laboratory Staff (1966) In: Green EL (ed) Biology of the laboratory mouse. McGraw-Hill, New York
24. Massry SG (1983) Pathogenesis of the anemia of uremia: role of secondary hyperparathyroidism. Kidney Int [Suppl 16] 24:S204–S207
25. Meyer MH, Meyer RA Jr, Gray RW, Irwin RL (1985) Picric acid methods greatly overestimate serum creatinine in mice: more accurate results with high-performance liquid chromatography. Anal Biochem 144:285–290
26. Morrison AB (1962) Experimentally induced chronic renal insufficiency in the rat. Lab Invest 11:321–332
27. Souhami RL (1972) Phagocytosis in uremic mice. J Reticuloendothelial Soc 12:463–472
28. Souhami RL (1973) Antibody production and catabolism in uremia. Br J Exp Pathol 54:380–387
29. Weber H, Kong Yann L, Bricker NS (1975) Effect of sodium intake on single nephron glomerular filtration rate and sodium reabsorption in experimental uremia. Kidney Int 8:14–20
30. Wolford ST, Schroer RA, Gohs FX, Gallo PP, Brodeck M, Falk HB, Ruhren R (1986) Reference range data base for serum chemistry and hematology values in laboratory animals. J Toxicol Environ Health 18:161–188

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