Urol Res (1983) 11:11–14

Urological Research

A Reproducible Model for Chronic Renal Failure in the Mouse

R. F. Gagnon and W. P. Duguid[1]

Montreal General Hospital Research Institute and the Departments of Medicine and [1]Pathology of the Montreal General Hospital, McGill University, Montreal, Canada

Accepted: October 21, 1982

Summary. Chronic renal failure was induced in mice using bilateral electrocoagulation of the renal cortex. Thermal injury via an angled point cautery was applied to the fully exteriorized right kidney. Following a 10-day interval the left kidney was injured in a similar fashion. The incidence of local complications was negligible and because of the limited blood loss, very small laboratory animals can be used. By restricting trauma to varying proportions of the visible cortex graded levels of injury were obtained. Thus this model can produce a uniform pattern of renal failure and will therefore facilitate the acquisition of information on the biological consequences of chronic uraemia.

Key words: Uraemia, Electrocoagulation, Renal cortex, Experimental uraemia, Chronic renal failure.

Introduction

Chronic renal failure has been produced successfully in the rat [1, 2] and in the dog [5] by causing thermal injury to the kidney surface. Because of the lack of haemorrhage and technical ease, this technique seems particularly suited for inducing renal failure in even smaller laboratory animals. Souhami [6] has rendered mice severely uraemic by repeated punctate cortical injury with a hot needle. For over a year, we have constantly practised electrocoagulation of the renal cortex, using mice and rats of all ages with a high success rate in the production of chronic renal failure. In this paper we are reporting in detail the method we have devised for electrocoagulation of the renal cortex in adult mice.

Materials and Methods

Male $C_{57}Bl_6$ mice purchased from Jackson Laboratories (Bar Harbor, Maine, USA) and maintained in our facilities were 5 weeks old at the start of the experiments. Prior to the study, experience was obtained with electrocoagulation, using a foot-operated single point cauterizer angled at 30° (Hyfrecator, Model X-712, The Butcher Corp., Los Angeles, USA) and set at 25 mV. Once satisfactory discrete punctate superficial cortical lesions were obtained consistently, studies of the effects of the procedure on renal function and morphology were initiated.

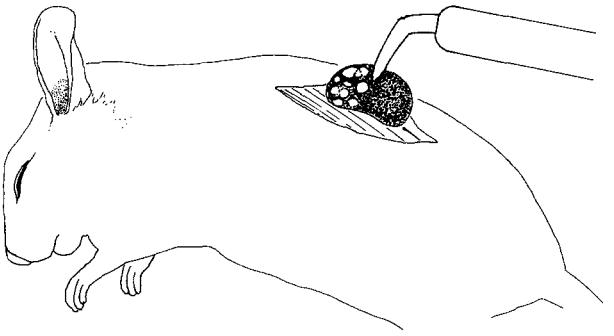

Fig. 1. Schematic illustration of surgical preparation of mice. Cautery tip in position over medial aspect of fully exteriorised left kidney with upper cortex already electrocoagulated

Mice were anaesthetized with ether, shaved and then placed on their left side. The right kidney was approached through a 2 cm long lumbar incision. Inserting two wet cotton buds on either side of the renal hilum, the kidney was pushed outwards and fully exteriorized. It was then freed from its attachments to surrounding tissues by fine dissection leaving the renal capsule intact. The animal was immediately transferred to the metallic plaque of the cauterizer and with direct exposure of the ventral aspect of the kidney, electrocoagulation of this surface was performed systematically from superior to inferior poles (Fig. 1). The cautery tip was applied to all areas of exposed cortex except for a 2 mm band of intact tissue around the hilum. The punctate lesions were spaced 2 mm apart. They were 1 mm deep and surrounded by a blanched area. Eye protection was worn because of the risk of fire in the event of a spark from the cautery point in making contact with tissue in the ether-enriched atmosphere. The kidney was held in an exteriorised position throughout in order to prevent injury to the hilum and related structures. In turn, the same procedure was applied to the dorsal surface. Following electrocoagulation, the kidney was guided back into the renal fossa. The deep tissue layers were approximated with a 3–0 silk running suture and the skin was closed with metal clips. Bleeding was generally minimal. The entire procedure occupied less than 5 min. Post-operative mortality (mainly due to excessive anaesthesia) was

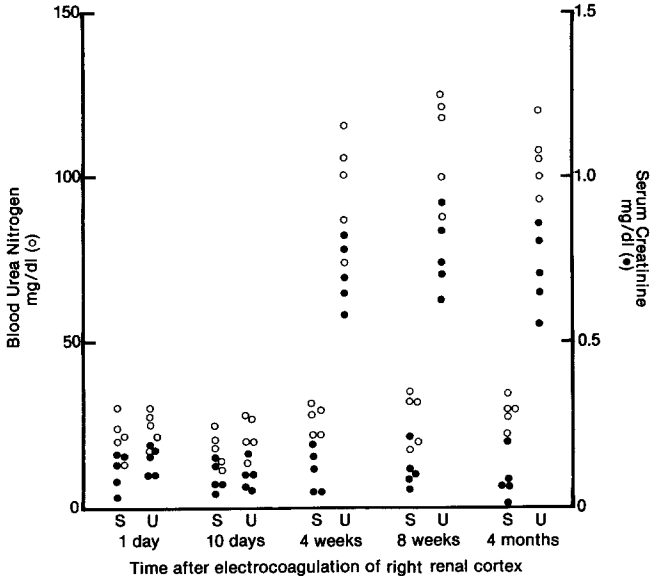

Fig. 2. Deterioration in renal function following bilateral electrocoagulation of the renal cortex. *S:* sham-operated animals, right kidney electrocoagulation on day 0 and removal of left kidney capsule on day 10; *U:* sequential electrocoagulation of right and left renal cortices on days 0 and 10, respectively

reduced to below 2%. Ten days later, the left renal cortex was submitted to identical treatments. Alternatively, in sham-operated animals, the left renal capsule was removed. Subsequently, all animals were fed on Purina chow pellets containing 11% protein and a 5% solution of glucose in water freshly prepared every other day.

At various times following the first and second electrocoagulations the animals were killed, blood was drawn by cardiac puncture and blood urea nitrogen (BUN) and serum creatinine values were determined by an IL9-autoanalyzer (Instrumentation Laboratory Inc., Lexington, Mass, USA.). For light microscopy, transverse blocks of kidney tissue obtained at the level of the hilum were fixed in 10% buffered formalin, cut and stained with hematoxylin and eosin.

Results

Mice which survived the initial surgery lived until their designated date of sacrifice up to 4 months post-operatively. Autopsy performed in all instances revealed no gross intraperitoneal abnormalities. The electrocoagulated portion of the kidney was gray-coloured, atrophied and often adherent to surrounding structures.

Renal Function

The results of renal function indices in mice made uraemic by bilateral electrocoagulation of the renal cortex and in sham-operated controls are given in Fig. 2.

Sham surgery had no demonstrable effect on the renal function of control animals. BUN and serum creatinine values of the sham-operated animals remained within normal limits throughout.

In contrast, bilateral electrocoagulation of the renal cortex had a marked deteriorating effect on renal function. Four weeks after bilateral electrocoagulation of the renal cortex, the BUN and serum creatinine values have increased considerably and remained so for several weeks afterwards. The level of rise of renal function indices in these mice would correspond to renal failure of moderate to severe degree.

Renal Morphology

Photomicrographs illustrating the progression of the thermal lesion are presented in Fig. 3.

In sections examined from the area immediately after injury the areas of coagulation can be defined due to elongation and distortion of nuclei. The outer 20% of cortex is involved. At 1 h it is evident that both glomeruli and tubules are involved. There is loss of basophilia of the cytoplasm and pyknosis of nuclei indicating necrosis. These changes progress and at 5 h there is absence of nuclear staining and the appearance is that of a patchy cortical necrosis. In the adjacent zone fatty change in the tubular epithelial cells is evident.

At 24 h there is extension of the cortical necrosis circumferentially and also deeper into the cortex. A mild inflammatory reaction is noted in the renal capsule but there is no inflammatory cellular exudate in the renal parenchyma.

Between 24 and 48 h there is progressive vacuolation of tubules and evidence of necrosis and repair. Some tubules contain proteinaceous casts. Repair in tubules is maximal at 72 h. At this time there is now a mild inflammatory reaction in the renal parenchyma surrounding the infarcted areas of the cortex.

At 10 days there is a marked inflammatory reaction around the kidney with early fibrosis. There is now loss of nephrons with replacement fibrosis and narrowing of cortex. The latter changes are progressive and at eight weeks the damaged cortex is replaced by a thin layer of fibrous tissue in which there is dystrophic calcification. There is now evidence of intra-renal hydronephrosis.

Discussion

As described in this report bilateral electrocoagulation of the renal cortex appears a satisfactory technique for the induction of chronic renal failure in mice. The severity of the damage is sufficient to give rise to advanced and stable renal failure from 4–6 weeks after the thermal injury. Where more severe chronic renal failure is desired, a contralateral nephrectomy can be performed at the second setting in lieu of electrocoagulation. The procedure has the advantages of rapidity and simplicity. Two major factors contributing to the success of the procedure are the use of an angled single point cauterizer to maximise visibility and exteriorization

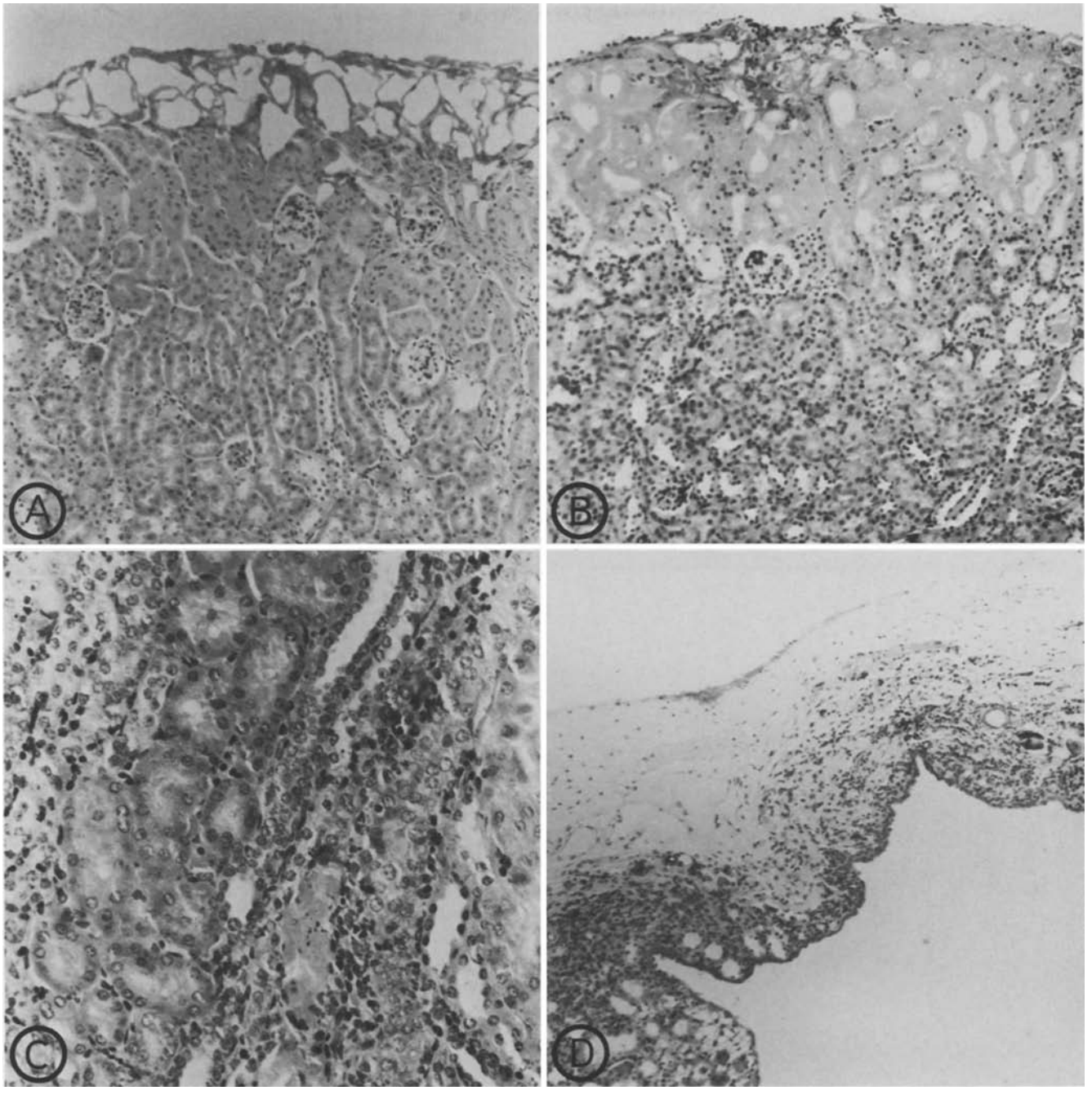

Fig. 3. Sequence of histological changes following electrocoagulation of mouse renal cortex. **A)** Immediately after electrocoagulation. Capsular oedema and nuclear distortion within cells of the outer cortex are seen (H&E x 135). **B)** 3 h post-electrocoagulation. Early patchy coagulative necrosis involving both glomeruli and tubules is present in the sub-capsular area. There is no inflammatory reaction (H&E x 110). **C)** 10 days post-electrocoagulation. The continuing necrosis and regeneration of tubules extend more deeply into cortex. There is minimal interstitial inflammatory reaction (H&E x 253). **D)** 11 weeks post-electrocoagulation. The previously damaged areas of cortex are atrophic and completely replaced by a thin rim of fibrous tissue. The atrophy has resulted in intrarenal hydronephrosis. Dystrophic calcification is also present (H&E x 110)

of the kidney to ensure thoroughness of the controlled injury.

When electrocoagulation of both kidneys is performed during the same operation, the animals die within 3 to 4 days in acute renal failure. The lesions are similar to those found in bilateral ischaemic cortical necrosis. Thus, staging the procedure allows time for some regeneration in the first kidney. The necessity of two operations may be considered a limitation of the technique but it is essential for the establishment of stable chronic renal failure. In addition, changes in the thymus and spleen have been demonstrated in rats following a single focal thermocoagulation of the kidney [3,

4]. More widespread use of the procedure is necessary to demonstrate any such untoward systemic effects.

Acknowledgements: The excellent technical assistance of Marie-Paule Gagnon and Fay Zeira is gratefully acknowledged. We thank Mrs. A. Esposito for typing the manuscript, Miss Margo Siminovitch for drawing the illustrations and Susan Moses for librarian assistance. This investigation was made possible through the unfailing support of Dr. Michael Kaye. Financial aid was provided by a grant (# MA-7479) from the Medical Research Council of Canada. R. F. Gagnon is the recipient of a scholarship from the Conseil de la Recherche en Santé du Québec.

References

1. Boudet NK, Mann PP, Sausse A, Funck-Brentano JL (1978) Experimental chronic renal failure in the rat by electrocoagulation of the renal cortex. Kidney Int 14:82–86
2. Bricker NS, Klahr S, Rieselbach RE (1964) The functional adaptation of the diseased kidney. I. Glomerular filtration rate. J Clin Invest 43:1915–1921
3. Grouls V, Helpap B (1978) DNA-synthesis in the rat thymus after focal thermolesions on abdominal organs. Res Exp Med (Berl) 173:229–237
4. 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
5. Shankel SW, Robson AM, Bricker NS (1967) On the mechanism of the splay in the glucose titration curve in advanced experimental renal disease in the rat. J Clin Invest 46:164–173
6. Souhami RL (1973) Antibody production and catabolism in uremia. Br J Exp Pathol 54:380–387

Dr. R. F. Gagnon
Division of Nephrology
2nd Floor, Livingston Hall
Montreal General Hospital
1650 Cedar Ave
Montreal, Quebec, H3G 1A4
Canada