Urological Research 7, 253-260 (1979)

Urological Research

Vascular Lesions in the Rat After Ligation of the Inferior Vena Cava Above the Renal Veins

Barbara Du Rietz, Rolf Ekman and Arne M. Olsson

Departments of Pathology, Clinical Chemistry and Urology, University of Lund, Sweden

Accepted: August 21, 1978

Summary. Raised renal venous pressure induced by ligation of the inferior vena cava above the renal veins in rats produces an essentially transient renal injury with electrolyte imbalance, phosphate retention but normal calcium levels and blood pressure. Medial necrosis and calcification of the aorta and the large arteries develop within a few days. Discrete arteritis - periarteritis focal myocarditis and visceral calcification are also seen. The parathyroid glands show signs of hyperfunction.

Key words: Renal venous congestion - Medial arteral necrosis - Calcification - Experimental hyperparathyroidism.

In a previous study of kidney morphology after renal venous stasis induced by suprarenal vena cava ligation (SRVCL), we noted, as an incidental finding, necrotizing and calcifying lesions in large arteries (8). SRVCL produces rapid changes in the serum electrolytes, temporary azotemia and functional and morphological renal lesions in the rat (1, 8). The severity and extent of the renal damage is to a large degree correlated with age, older rats showing more severe changes and higher mortality (8). The present work was undertaken to further investigate the vascular pathological-anatomical changes, and if possible to elucidate the pathogenesis.

MATERIAL AND METHODS

All experiments were performed on male Sprague-Dawley rats, weighing between 250 and 350 g, fed on pellets (Astra-Ewos) and water ad libitum during the entire experimental period.

Laparotomy was performed under ether anaesthesia by a midline incision. The vena cava was dissected free and ligated with a silk tie just above the renal veins (7). In 25 sham-operated animals only the dissection was performed. At 1, 2, 3, 4, 8, 16, and 32 days after the operation 3 SRVCL-operated and 3 sham-operated rats were again anaesthetised with ether and a further laparatory performed. The aorta was dissected free and punctured and the blood pressure was registered with an electromanometer (Siemens-Elema, Sweden), whereafter the animals were killed by exsanguination. Determinations of albumin, calcium, phosphate and alkaline phosphatase (S-ALP) were made at the routine Clinical Chemical Laboratory, University Hospital, Lund (2, 6, 16).

Autopsies were immediately performed on the sacrificed animals as well as on animals which succumbed spontaneously after caval ligation. The kidneys, adrenals, heart, liver and spleen were weighed. Specimens from these organs and from the lungs and stomach as well as the parathyroids, thyroid, thymus, aorta and striated muscle from the hind leg were immersed in Histofix® (Histolab, Sweden). All tissues were embedded in paraffin, sectioned and stained routinely with hematoxylin and erythrosin and von Kóssa's silver nitrate stain for calcium. Selected sections were stained by special methods including van Gieson, Periodic-Acid-Schiff (PAS) method and toluidine blue.

RESULTS

1. General Observations

In the SRVCL-operated rats there was a 25% decrease in body weight on the fourth day but they had returned to their pre-operative weights on

0300-5623/79/0007/0253/$01.60

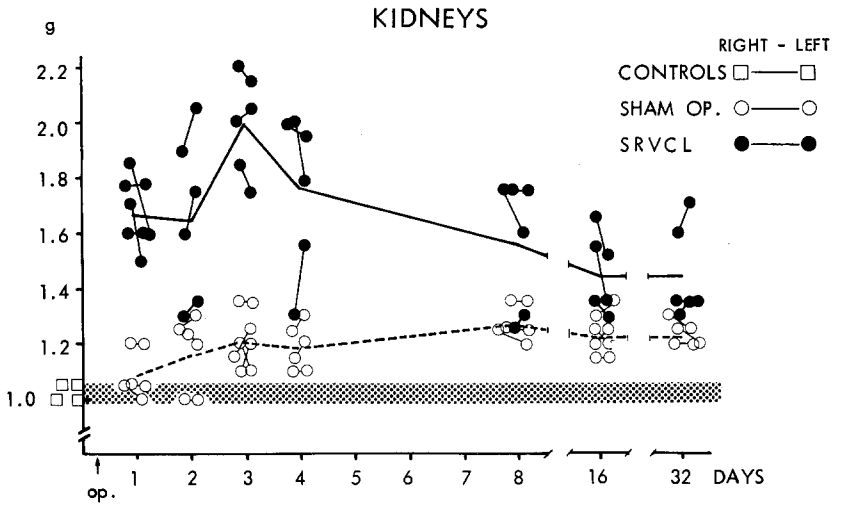

Fig. 1. Wet weight of the kidneys in controls (□), sham-operated (O) and SRVCL (●) animals. Mean values illustrated by lines

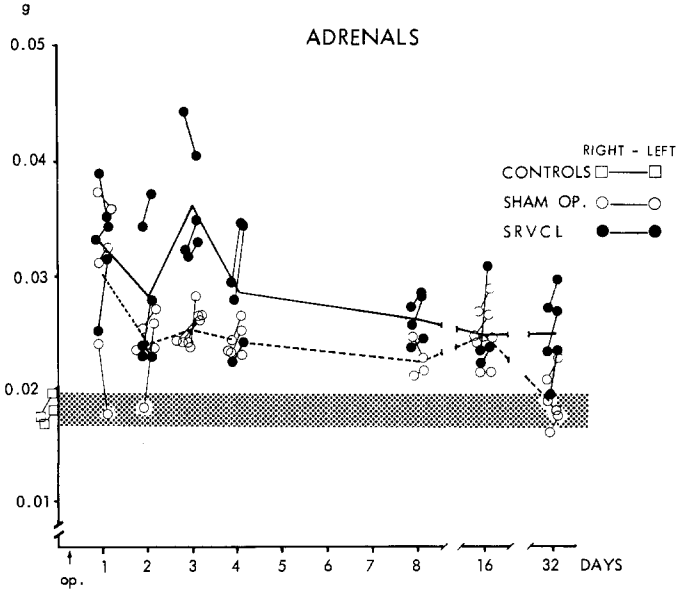

Fig. 2. Wet weight of the adrenals in controls (□), sham-operated (O) and SRVCL (●) animals. Mean values illustrated by lines

the 16th day. The sham-operated animals had a weight loss of 3-10%, achieving pre-operative weights on the fourth to the eight day. At 32 days the cava-ligated rats still weighed less than the sham-operated rats.

The wet weights of kidneys and adrenals are given in Figure 1-2. The kidneys of the cava-ligated rats were, at first, significantly heavier ($P < 0.001$) than those of the sham-operated. The weight difference decreased with time until at 16 and 32 days it was insignificant. While there were no side-differences between the kidneys, the left adrenals were significantly heavier ($P < 0.001$) than the right adrenals in both groups. The adrenals of the cava-ligated rats were significantly heavier ($P < 0.001$) than those of the sham-operated but after the first postoperative day a parallel weight decrease in both groups was seen. No difference in heart weight was seen. The mean liver weight at 1-16 days, was reduced up to 30% in cava-ligated compared to sham-operated rats, but at 32 days however, no weight differences were noted. There was significant reduction ($P < 0.001$) in spleen weight during the first postoperative week in the cava-ligated rats.

No hypertension was seen in cava-ligated, sham-operated and control animals.

The effect of SRVCL on the phosphate levels are shown in Figure 3. A 2-3 fold increase of the phosphate level was demonstrated during the first two days after operation and then returned after four days to pre-operative level. In the sham-operated rats the level was essentially constant. No differences were found in the serum levels of albumin, calcium and S-ALP between the SRVCL- and sham-operated animals.

2. Microscopic Findings

Kidneys. The renal lesions produced by ligation of the inferior vena cava above the renal veins have previously been reported in detail (7). The kidneys in the first two to three days after operation showed vascular congestion, degeneration and necrosis of tubules as well as hyaline and granular casts. The lesions were characteristically most extensive in the inner stripe of the outer medulla and the adjacent inner medulla. Necrosis of short segments of the proximal convoluted tubules in the outer cortex was also often conspicuous. On the second-third day there was regeneration and repair of the epithelium. Renal morphology returned to near normal after one to two months although slight interstitial fibrosis and scattered

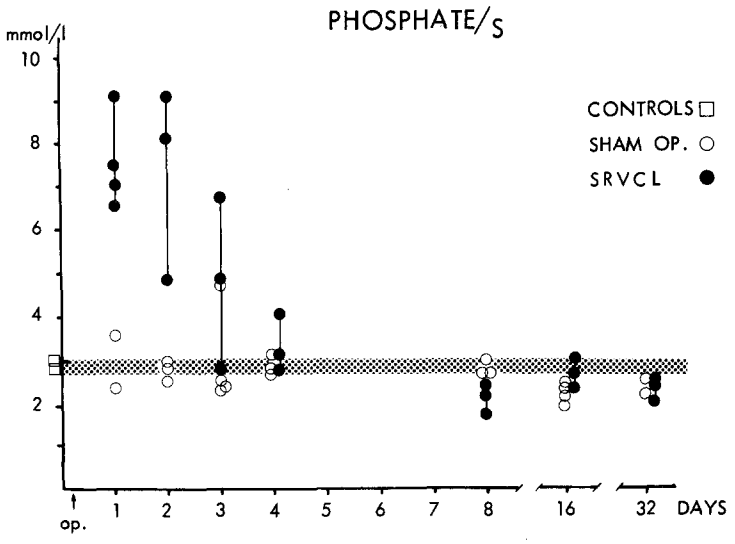

Fig. 3. Phosphate concentration in serum in controls (□), sham-operated (O) and SRVCL (●) animals

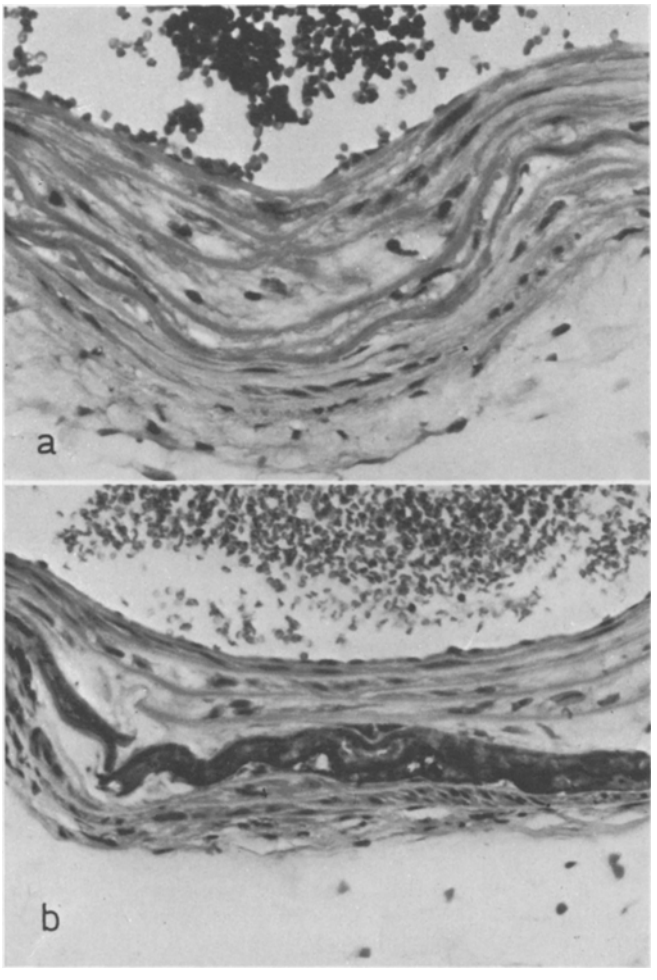

Fig. 4.a Aorta of a rat 3 days after caval ligation. The elastic laminae of the media are wrinkled, spread apart and finely encrusted with calcium. Many of the smooth muscle cells appear necrotic. Haematoxylin and erythrosin x 225. b Large parts of the aortic media are calcified in continuity. A calcified fracture is seen to the left. Sixteen days after caval ligation. Haematoxylin and erythrosin x 225

atrophic tubules were seen. Older rats recovered at a slower pace and with more extensive sequelae.

Deposits of calcium were found in varying amounts in all kidneys from the second day onwards. Calcified necrotic or atrophic tubules as well as intratubular calcified casts were most often seen in the outer medulla and the outer cortex or at the margins of infarctions. In a few instances small calcium deposits were noticed in the glomerular tuft and in Bowman's capsular epithelium. The calcium deposits, initially granular, after a few days appeared larger and more solid but otherwise seemed to persist unchanged. The calcified casts, though, were often surrounded by a syncytium of tubular epithelial cells and occasional giant cell-granulomas were seen.

Animals which succumbed spontaneously during the first two days showed more severe renal lesions. Eight of nine rats had medullary infarctions, bilateral in four cases.

Controls and sham-operated animals: Scattered calcified tubular casts were found in the renal papillae of one rat out of 25, otherwise no histological changes were noticed.

Aorta and the Large Arteries. The earliest changes in the aorta were observed on the third day after SRVCL. Fine granules of calcium were seen in and along the elastic laminae of the media and in the interlaminar matrix. Simultaneously the smooth muscle cells of the affected parts of the media appeared necrotic (Fig. 4a). The calcification, though variable, increased during the first weeks. The least affected aortas showed a patchy encrustation of the elastic fibers with calcium. In those with more severe lesions the elastic laminae were heavily impregnated and separated by accumulations of metachromatic substance and calcium. In advanced cases large parts of the me-

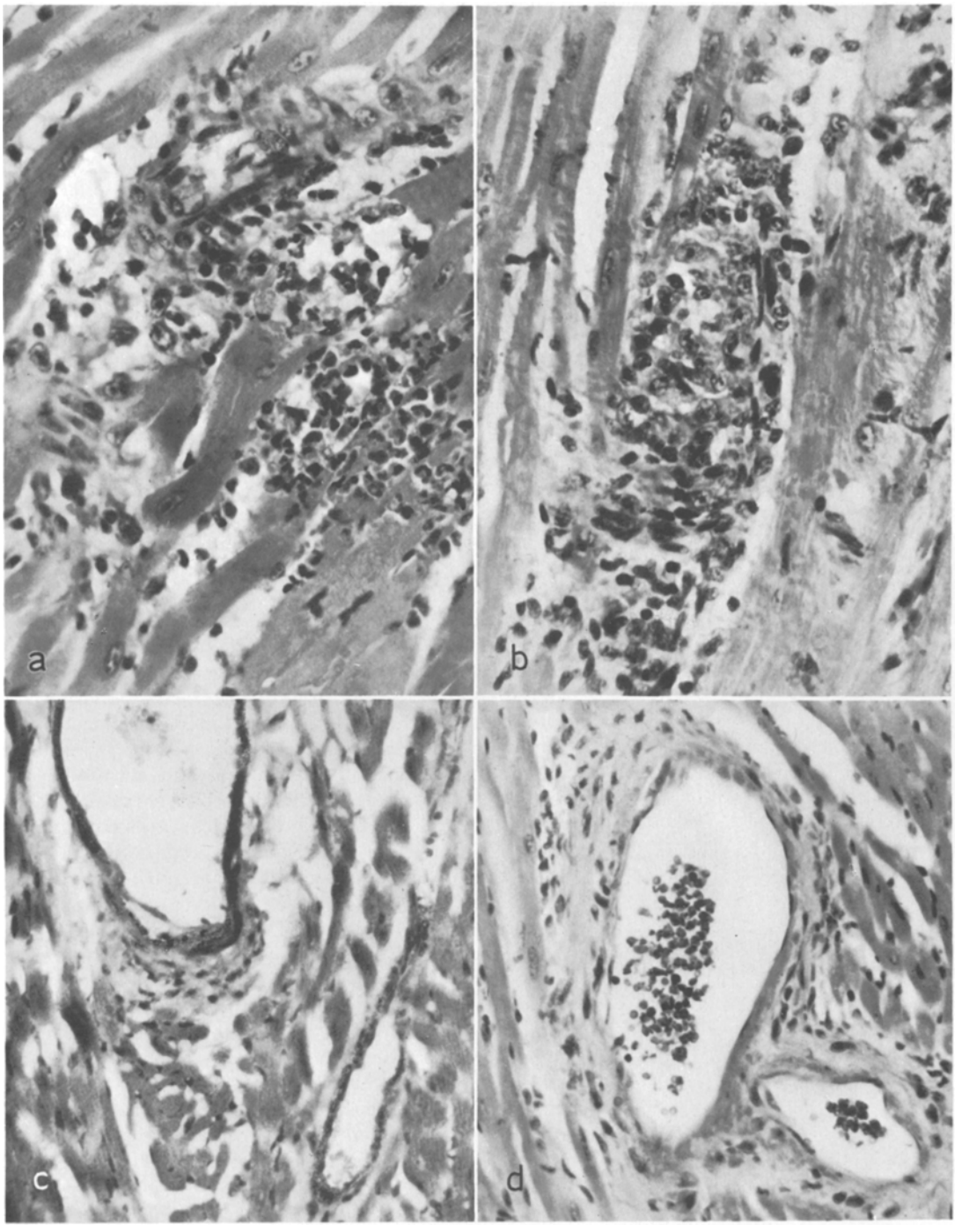

Fig. 5. a Focal myocarditis with destruction of muscle cells and infiltration of leucocytes and histocytes. Three days after caval ligation. Haematoxylin and erythrosin x 400. b Arteritis - periarteritis in myocardium of a rat killed four days after caval ligation. Haematoxylin and erythrosin x 400. c and d The small coronary arteries are dilated and in parts finely encrusted with calcium. Perivascular fibrosis and inflammation are apparent. Sixteen days after caval ligation. c - von Kossa x 250; d - Haematoxylin and erythrosin x 250

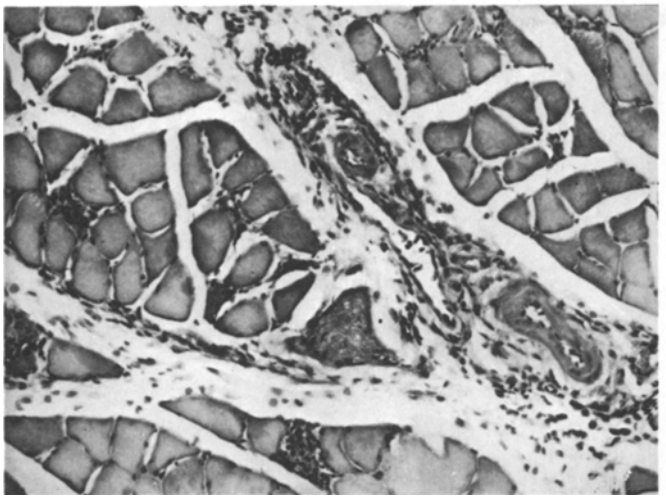

Fig. 6. Striated muscle from the hind leg of a rat three days after caval ligation. There is arteritis-periarteritis with interstitial inflammatory changes and scattered necrotic muscle fibers with a cellular reaction. Haematoxylin and erythrosin x 120

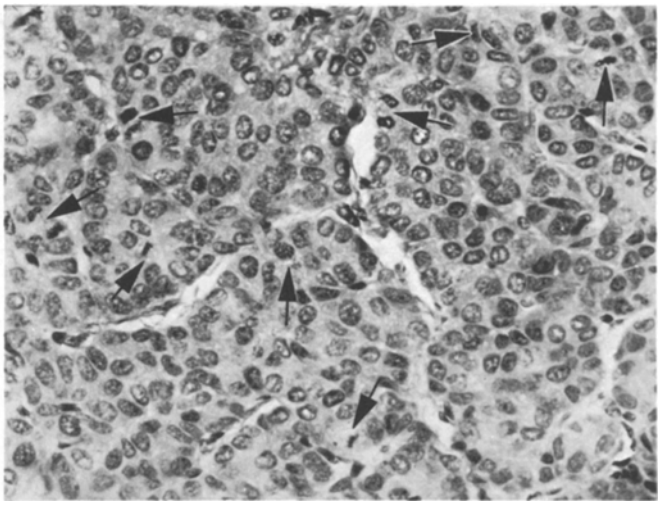

Fig. 7. Parathyroid gland of a rat four days after suprarenal caval ligation. The cells are hyperplastic and numerous mitoses (arrows) are seen. Haematoxylin and erythrosin x 320

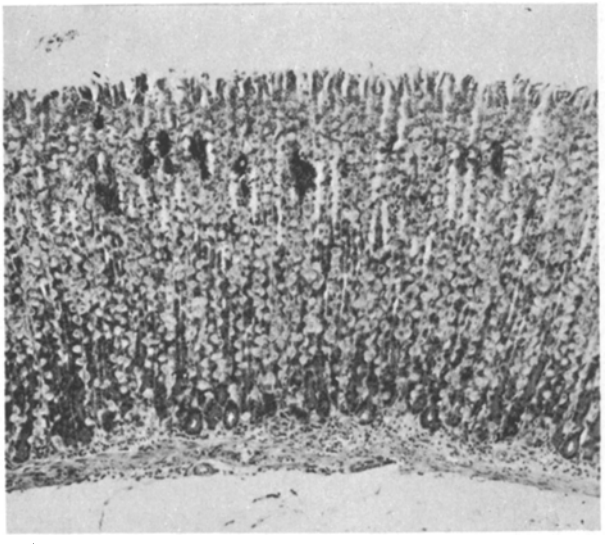

Fig. 8. Dense calcium deposits in the lamina propria of the gastric mucosa. Sixteen days after caval ligation. von Kossa x 70

dia were calcified in continuity and fragmentation was seen (Fig. 4b). There was no inflammation except occasionally when the intima or the adventitia was damaged by fractured calcifications. In one animal on the 32nd day small areas of chondroid metaplasia in the media were noticed.

Major branches of the aorta showed similar lesions. Changes were most often found in the carotid arteries. In severely affected animals calcium deposits were also found in the pulmonary arteries.

Small Arteries and Arterioles. Arteritis and periarteritis - mostly discrete - occurred in about 2/3 of the animals after SRVCL. It was most consistently noticed in skeletal and cardiac muscle and was observed as early as the second day (Fig. 5b). In the early stages calcification was seldon seen. The perivascular interstitial inflammation was sometimes pronounced and extended into adjacent muscle fibers, which showed degenerative changes. In the later stages the inflammatory reaction in the muscle had subsided though arteritis with signs of healing and calcification were seen (Fig. 5c + d).

Heart and Skeletal Muscle. In addition to arteritis and periarteritis, a focal myocardial and skeletal muscle necrosis, accompanied by leucocytic and mononuclear infiltration was seen in a few animals on the 2nd-4th day after SRVCL (Fig. 5a). Later, focal fibrosis and small calcium deposits in isolated muscle fibers became a rare finding. In the skeletal muscle there was evidence of regeneration with thin fibers containing basophilic sarcoplasm and many large nuclei (Fig. 6).

Parathyroids. In all but a few animals two parathyroid glands were found; in one rat there were three glands and in another four glands. On the 2nd day after SRVCL both the nucleus and cytoplasm of the chief cells were increased in size. The cellular hypertrophy persisted over the next two days. On the 3rd and 4th days there were signs of rapid cell proliferation with numerous mitoses (between 10-45 mitoses in each transverse section of every gland) (Fig. 7). By the eighth day the mitotic activity had subsided and cellular hypertrophy was

no longer evident. The parathyroid glands were enlarged in the experimental group from the 3rd day onward, the size being estimated from the microscopic cross-section.

Adrenals. During the first four postoperative days the adrenals from cava-ligated animals showed varying degrees of vascular congestion and interstitial oedema. In several glands there were small areas of focal necrosis in the zona fasciculata and in two cases small unilateral haemorrhagic infarcts - also limited to the zona fasciculata - were found. Cells in all zones often had an abundant foamy cytoplasm, and varying numbers of intracytoplasmic hyaline PAS-positive droplets were noticed in both cortex and medulla. Between the 2nd and 4th day a few mitotic figures were also found in all adrenals. At 8, 16 and 32 days there were no consistent differences between cava-ligated and sham-operated animals.

Adrenals from animals succumbing spontaneously during the first two days after surgery showed the same changes as rats killed at the same time though unilateral cortical infarcts in all cases were more common.

Changes in Other Organs. Calcification in the lungs, the stomach (Fig. 8) and the thymus was seen in more than half of the cava-ligated animals to various degrees from the second day onward. In general the rats with the most severe aortic lesions also showed the most extensive visceral calcifications.

The spleen in the first four to eight days after SRVCL was contracted and bloodless, but thereafter appeared normal. No changes were seen in the aorta, large and small arteries, cardiac and skeletal muscle, parathyroids or other organs in sham-operated animals or controls.

DISCUSSION

Vascular lesions develop after many different renal manipulations. The so-called obstructive nephropathy induced by administering an excessive amount of a poorly soluble sulphonamide (Na-acetyl sulphathiazole) to rats produces a type of vascular lesion very similar to that found after SRVCL, namely medionecrosis and calcification in the aorta and the large arteries. The small arteries and arterioles showing only minimal lesions. The renal as well as the cardiovascular lesions are fully developed within five to six days (5, 19, 20, 21). Bilateral nephrectomy gives rise to the same type of vascular injury but the animals succumb after 3-5 days (5, 21). Partially nephrectomised rats develop arterial lesions only after several months (10, 11, 24, 27, 30).

A marked rise in serum creatinine and inorganic phosphate are the regular findings after SRVCL as in other experimental models inducing acute renal failure. After SRVCL the total serum calcium concentration, though not measured during the first postoperative hours, was normal. Ionized calcium may have been lowered, however, thereby stimulating the parathyroids. In obstructive nephropathy the calcium concentration is subnormal during the first week, but returns to normal after 10-15 days (21). Different investigators agree that there is a reduction in circulating ionized calcium in nephrectomised rats though their opinion differ concerning the total calcium concentration (17, 28, 32). Animals subjected to adrenalectomy as well as nephrectomy had increased calcium levels in comparison with nephrectomy only and in rats made uraemic by partial nephrectomy, some authors observed increased while other found normal or subnormal calcium level. These differences may be due to several factors; experimental model, species, and strains of experimental animals, sampling and analysing methods etc. Further, serum calcium estimation at a particular moment may not afford sufficient information about the calcium metabolism or the level of ionized calcium needed to stimulate parathyroid activity.

Results of blood pressure studies after different renal manipulations vary as much as much as those of serum calcium. Obstructive nephropathy in rats causes marked but transient hypertension (5, 19). Bilateral nephrectomy also gives rise to hypertension but when the parathyroids are removed prior to nephrectomy the renoprival hypertension appears to be abolished and the emergence of cardiovascular necrosis is also prevented. It is however stated that the hypertension is not required for development of the cardiovascular lesions (21). We found that suprarenal caval ligation on rats and rabbits did not alter the blood pressure (25). There are however other reports of hypertension in about 50% of different experimental animals after raised renal venous pressure (26), but no evidence of hypertension as a cause of vascular lesions is presented.

Hypertrophy and hyperplasia of the parathyroid glands as well as adrenal cortical hypertrophy are seen after administration of Na-acetylsulphathiazole and bilateral and subtotal nephrectomy. SRVCL too induces parathyroid hypertrophy and hyperplasia but no sign of adrenal hypertrophy. Though a few mitotic figures are seen in all adrenal glands there are no consistent differences between cava-ligated and sham-operated rats. Parathyroidectomy in rats made uraemic by partial nephrectomy fully protects against medial calcification and prevents to some degree the development of medial necrosis (10). According to Lehr and Martin the removal of the parathyroid glands prior to nephrectomy or administration of sulphathiazole provides complete protection against development of the cardiovascular lesions and smooth muscle necrosis. A similar protective

effect is achieved by adrenalectomy. Lehr and Martin suggested that autointoxication with hormone from excessively secreting parathyroid glands is the main cause of the emergence of vascular and muscular damage in rats with renal injury. They also postulated that the enhanced parathyroid activity is mediated by the adrenals (22). This was also supported by Hansson (14).

Hypervitaminosis D in rats and rabbits also induces lesions in the aorta and the larger arteries (9, 12, 13). The parathyroid glands are however normal in size according to Hass (15) and removal of the parathyroids does not interfere with the development of the severe calcifications (21).

Necrotizing vascular disease especially of the polyarteritis nodosa type is a different type of vascular lesion principally involving the small arteries and arterioles and is seen following renal infarctions (18, 23). Other experimental techniques which produce this type of vascular injury are perinephritis induced by wrapping the kidney in silk and unilateral nephrectomy combined with the administration of NaCl and DOCA. In these experimental models no lesions are found in the aorta and the large arteries (3, 29). SRVCL-rats also show changes in the small arteries and arterioles but only in 2/3 of the animals and in most rats the lesions are discrete. Vascular lesions of the periarteritis nodosa type is probably not dependent on parathyroid secretion. This assumption is supported by several studies. For example Like and Orbison, found that cauterisation of the parathyroid glands does not prevent vasculitis induced by renal infarctions (23). Adrenalectomy did, however, distinctly diminish the vascular disease (18). Evidence also exists that the vasculitis appearing in minor vessels is a hypersensitivity reaction and the result of circulating immune complexes (4, 31).

The experimental evidence to date indicates that vascular lesions induced by renal injuries are the result of a combination of several disturbances, e.g. electrolyte changes, altered concentration of adrenal and parathyroid hormones, circulating immune complexes and perhaps sometimes hypertension. Variations in these factors may be responsible for the two major different vascular lesions seen in our experiments, i.e. calcifying medial necrosis and vasculitis of the periarteritis nodosa type. Further studies may more precisely evaluate the influence of each factor for the development of the vascular disease.

REFERENCES

1. Bengmark, S., Olsson, A. M.: Acute obstruction of the suprarenal vena cava in the rat. II changes in electrolytes, urea, creatinine and acid-base balance. Acta Chirurgica Scandinavica 140, 546 (1974)
2. Bessey, O. A., Lawry O. H., Brock, M. J.: Committee on enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology. Scandinavian Journal of Clinical and Laboratory Investigation 33, 291 (1974)
3. Campbell, W. G., Jr., Santos-Buch, C. A.: Widely distributed necrotic arterial lesions induced in rabbits by experimental renal alterations. V. Light microscopie studies of early lesions of arterial, cardiac, and skeletal muscle. Laboratory Investigation 15, 1856 (1966)
4. Campbell, W. G., Jr., Santos-Buch, C. A.: Widely distributed necrotic arterial lesions induced in rabbits by experimental renal alterations. VI. Demonstration of antigenic determinants of -globulin and of thrombinclottable plasma protein in arterial lesions: Uptake of guinea pig complement in vitro. Laboratory Investigation 15, 1870 (1966)
5. Churg, J., Paterson, N. J.: Renal and renoprival vascular disease in the rat. Archives of Pathology 75, 547 (1963)
6. Doumas, B. T., Watson, W. A., Biggs, H. G.: Albumin standards and the measurement of serum albumin with bromcresolgreen. Clinica Chimica Acta 31, 87 (1971)
7. Du Rietz, B., Ekman, R., Olin, T., Olsson, A. M.: Experimental normocalcaemic hyperparathyroidism induced by renal venous congestion. Urological Research 5, 109 (1977)
8. Du Rietz, B., Olsson, A. M.: Renal changes in rats after ligation of the inferior vena cava above the renal veins. Acta Chirurgica Scandinavica 140, 556 (1974)
9. Eisenstein, R., Zeruolis, L.: Vitamin D-induced aortic calcification. Archives of Pathology 77, 27 (1964)
10. Ejerblad, S., Eriksson, J.: Morfologiska artärförändringar vid klinisk och djurexperimentell uremi. Acta Societatis Medicorum Suecanae. 84, 1975 (in Swedish)
11. Giese, J.: Acute vascular disease caused by severe renal ischemia. Acta Pathologica 56, 399 (1962)
12. Gillman, J., Gilberg, C.: Calcium, phosphorus and vitamin D as factors regulating the integrity of the cardiovascular system. Experimental Medicine and Surgery 14, 135 (1956)
13. Gillman, T., Grant, R. A., Hathorn, M.: Histochemical and chemical studies of calciferol-induced vascular injuries. British Journal of Experimental Pathology 16, 1 (1960)
14. Hansson, C. G.: Effects of adrenalectomy on the parathyroids in nephrectomized rats. Acta Endocrinologica 51, 350 (1966)
15. Hass, G. M., Trueheart, R. E., Taylor, C. B., Stumpe, M.: An experimental histologic study

of hypervitaminosis D. American Journal of Pathology 34, 395 (1958)
16. Itaya, K., Ui, M. : A new micromethod for the colorimetric determination of inorganic phosphate. Clinica Chimica Acta 14, 361 (1966)
17. Jastak, J. T., Morrison, A. B., Raisz, L. G. : Effect of renal insufficiency on the parathyroid gland and calcium homeostasis. American Journal of Pathology 215, 84 (1968)
18. Koletsky, S. : Necrotizing vascular disease in the rat. Archives of Pathology 59, 312 (1955)
19. Lehr, D., Churg, J., Milora, R. : The production of experimental arteriosclerosis and some factors important in its prevention. Bulletin New York Medical College 15, 130 (1952)
20. Lehr, D., Churg, J. : Human and experimental arteriosclerosis. Journal of the Mount Sinai Hospital, New York 19, 106 (1952)
21. Lehr, D. : Causative relationships of parathyroid hormone to renogenic and renoprival cardiovascular disease. Annals of the New York Academy of Sciences 72, 901 (1959)
22. Lehr, D., Martin, C. R. : Prevention of severe cardiovascular and smooth muscle necrosis in the rat by thyro-parathyroidectomy. Endocrinology 59, 273 (1956)
23. Like, A. A., Orbison, J. L. : The parathyroid and experimental vascular necrosis in the rat. Archives of Pathology 66, 739 (1958)
24. Morrison, A. B. : Experimentally induced chronic renal insufficiency in the rat. Laboratory Investigation 11, 321 (1962)
25. Olin, T., Olsson, A. M. : Renal blood flow and function following suprarenal ligation of the inferior caval vein. An experimental study in the rabbit. Scandinavian Journal of Urology and Nephrology 8, 54 (1974)
26. Pytel, A., Arabi, A., Volpyan, E. L. Sheshina, N. P. : Experimental substantiation of arterial hypertension induced by upset of the venous outflow from the kidney. Eksperimental' naya Khirurgiya i Anesteziologiya 18, 42 (1975)
27. Rather, L. J. : Experimental aortic disease in albino rats following renal operations. Proceedings of the Society for Experimental Biology and Medicine 85, 285 (1954)
28. Schönau-Jörgensen, F. : An investigation of the early hypercalcemia seen after nephrectomy in rats. Acta Endocrinologica 69, 60 (1972)
29. Selye, H. : The general adaption syndrome and the diseases of adaption. Journal of Clinical Endocrinology 6, 117 (1946)
30. Shimamura, T. : Ultra-structural studies of the medial aortic sclerosis in rats with experimentally induced chronic renal disease. Experimental and Molecular Pathology 13, 79 (1970)
31. Svendsen, U. G. : The role of thymus for the development and prognosis of hypertension and hypertensive vascular disease in mice following renal infarction. Acta Pathologica et Microbiologica Scandinavica, Section A 84, 235 (1976)
32. Talmage Roy, V., Toft, R. J., Davis, R. : Parathyroid activity in nephrectomized rats. Texas Reports of Biology and Medicine 18, 298 (1960)

Barbara Du Rietz
Department of Pathology
University of Lund
S-221 85 Lund, Sweden