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# INFLUENCE OF OBSTRUCTED RENAL LYMPH FLOW ON EFFECTS OF A NEPHROTOXIN ON RENAL FUNCTION

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*ABSTRACT*

*To study the influence of disrupted renal lymph drainage on the effects of a nephrotoxin, uranyl acetate (0.028 to 0.05mg/kg body weight) was injected into dogs the same day that the renal lymphatics were ligated and transected bilaterally. Larger doses produced effects which masked the influence of lymphatic ligation; a control group receiving uranyl acetate underwent a sham ligation of renal lymphatics. In renal function studies at 1, 3, 8 and 12 weeks after surgery and uranyl acetate, the urea, creatinine, PAH and electrolyte clearances of the "ligated" dogs were lower than those of the "non-ligated" shams. Daily clearances of urea and creatinine of the ligated were also lower than those of the shams. Urinary volume and protein excretion were also greater in the ligated subjects although elevated in both groups. The significance of the differences in the daily electrolyte clearances was less convincing. Renal lymphatic ligation with uranyl acetate injection produced greater changes than either lymphatic ligation or uranyl acetate alone. That the changes were not permanent or did not lead to chronic renal disease was probably due to regeneration of lymphatics, development of alternate routes of lymph flow and/or the small dose of uranyl acetate used to demonstrate the influence of lymphatic dysfunction on response to the nephrotoxin.*

Demonstration that disordered lymph flow is an important factor in the development or manifestation of diseases of organs produced by other agents can be a valuable contribution to a better understanding of the pathogenesis of disease. Evidence exists that some pathological conditions are not produced by a single etiological agent, but develop if there is also an ongoing disturbance of lymph flow (1-5). For example, opinion is divided whether all edema relates to some form of inadequate lymphatic function, whether it be overload of the lymphatic transport capacity, interference with lymphatic uptake by inflammation or congenital or acquired impedance to lymph flow by absence or hypoplasia of lymph vessels, stenosis, obstruction, or valvular incompetence. Some propose that ascites of cirrhosis is the end result of lymphatic insufficiency (5). Kline et al report that obstruction of cardiac lymphatics is associated with pathological findings similar to endocardial fibroelastosis (1). They also report that experimental animals with disordered cardiac lymph flow are more susceptible to induced bacterial endocarditis than controls (2). In each of these examples a primary factor is present, but disordered lymphatic function seemingly contributes to the manifestations of the disease state.

Although there are pertinent reports on renal lymphatics, relatively little information exists on the role of impaired lymph flow as a

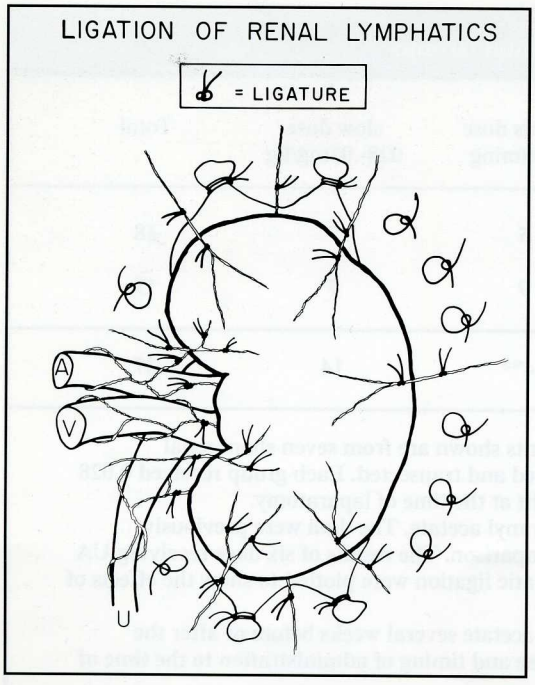


*Fig. 1. Diagram of location of the capsular and hilar renal lymphatics which were either ligated and transected bilaterally or sham-ligated.*

contributing factor to renal dysfunction of another primary etiology. J.J. Murphy *et al* reported that obstruction of the renal lymphatics appears to render the kidney more susceptible to infection by circulating microorganisms (3). We observed that obstruction of major lymphatic trunks in dogs is associated with an increased incidence of infection and inflammation of the kidney (6,7). Mechanical obstruction of major lymph channels or renal lymphatics produced transient changes in renal function but more permanent histological and ultrastructural changes (6). Clinically, only some patients with glomerulonephritis manifest the nephrotic stage or progress to the chronic stage, and not all subjects with renal disease exhibit the same severity of nephropathy or systemic manifestations from the same primary etiology. It appears, therefore, that more than one factor is necessary to produce noteworthy manifestations of renal disorders.

The lymphatic system serves as an accessory transport system for fluids and electrolytes and as a primary route for return of proteins and large molecules to the bloodstream (8). Lymphatics are frequently involved in inflammatory and reactive processes of organs and the vascular system which results in disruption of lymph flow. Therefore, it seemed reasonable that if there were an insult to the kidney which itself did not cause progression to an advanced stage, then, conceivably an accompanying disturbance of lymphatic drainage might contribute to the progression of renal disease. If this phenomenon was demonstrated, it could lead to attempts to develop therapeutic measures directed at correction of this defect. Thus, this project to study the effects of multiple noxious procedures (one being interference with renal lymph flow) on the development of renal dysfunction and pathology was conceived.

## *MATERIALS AND METHODS*

Thirty-seven non-pregnant worm-free female mongrel dogs were studied in pairs. One group had renal lymphatics ligated bilaterally and transected (henceforth termed "ligated"). The other had a sham-ligation laparotomy (henceforth "sham"). After an abdominal incision, the kidneys were exposed separately. Capsular and hilar lymphatics were identified and carefully isolated under magnification. Two ligatures of fine silk were placed around each lymph vessel. For the "ligated", these were tied and the lymphatic vessel transected *(Fig. 1)*. For the "sham", manipulations were the same except that only one silk strand was placed under the lymph vessel and then removed without tying. An attempt was made to minimize injury to adjacent small vessels. The ureters were left intact. Because identification of renal nerves was extremely difficult, it is possible that neural elements were included in the ligatures. Each dog ("ligated" and "sham") received intravenous injection of uranyl acetate (9-12). Groups consisted of variations in the dose of

**TABLE 1: Experimental Groups**

| | No uranyl* acetate | High dose 0.2-1.0mg/kg | various dose and timing | slow dose 028-.05mg/kg | Total |
|---|---|---|---|---|---|
| No Ligation | 7 | 6 | 5 | 7 | 18 |
| Ligation | 16 | 3** | 9 | 7 | 19 |
| Total | 23 | 9 | 14** | 14 | 37 |

Thirty-seven dogs were used in this study. The results shown are from seven sham renal lymphatic ligated and seven with renal lymphatics ligated and transected. Each group received 0.028 to 0.05 mg uranyl acetate (UA) per kilogram body weight at the time of laparatomy.

*Seven sham and 16 ligated dogs did not receive uranyl acetate. The data were previously reported (6) and are referenced here for purposes of comparison. The results of six dogs receiving UA doses from 0.2 to 1.0 mg/kg body weight without lymphatic ligation were plotted to show the effects of high dose of uranyl acetate alone *(see Fig. 2)*.

**Seventeen dogs received various doses of uranyl acetate several weeks before or after the laparotomy in order to establish the appropriate UA dose and timing of administration to the time of experimental preparation (operation).

uranyl acetate and in the temporal relationship of the administration of the nephrotoxin to the operative procedure to determine the most appropriate protocol to demonstrate the influence of renal lymphatic ligation. The preliminary studies indicated that the groups receiving 0.028 or 0.05mg/kg body weight of uranyl acetate intravenously the same day as surgery were most appropriate for that purpose *(Table 1)*. Larger doses of uranyl acetate produced toxic effects which masked the influence of renal lymphatic ligation *(Fig. 2)*.

Each dog was placed in a metabolic cage for daily collection of urine for a control period of from 2 to 6 weeks. Venous blood was obtained 3 times a week for a hemogram, plasma protein concentration, levels of urea nitrogen, creatinine, sodium, potassium, chloride, and osmolarity. Daily urine determinations included microscopic examination, volume, specific gravity, glucose, excretion of protein, urea, creatinine, sodium, potassium, chloride and osmolarity. All samples were run in duplicate.

The data were calculated to determine average daily clearance of creatinine, urea, sodium, potassium and chloride. At least two renal clearance studies were performed during the control period.

After injection of uranyl acetate, each dog was followed for approximately three months in the same manner as during the control period with periodic clearance studies. The averages of those performed at approximately 1 and 3 weeks and 2 and 3 months are reported here.

Standard clearance procedures were employed in the renal function studies with the dogs under pentobarbital anesthesia. Using an infusion pump, isotonic sodium chloride solution was administered at a constant rate of 0.5ml/kg/min for 150 minutes. Priming doses of creatinine and paraaminohippurate (PAH) were administered at the start of the infusion period and added to the solution infused in amounts calculated to maintain constant serum concentrations. After

a 30 minute equilibration period to allow stabilization of plasma PAH and creatinine, urine and blood were collected for 8 clearance periods of 15 minutes each. Serum and urine were analyzed for sodium, chloride, potassium, creatinine, urea, PAH and osmolarity, and the rate of urine flow was recorded.

The data from the eight 15 minute periods were averaged and calculated to study concentration of substances in plasma and urine, rates of urinary excretion, renal blood flow, glomerular filtration rate, tubular reabsorption, concentrating and diluting capacity.

Because there was variation in absolute values from dog to dog, the average of each group was expressed for comparative purposes as a percentage of the mean value of each group during the control pre-procedure period. This method provided each ligated dog with its own control and comparison with a similar self-controlled sham.

## *RESULTS*

### *Renal function clearance studies*

The administration of the low dose uranyl acetate (0.028-0.05mg/kg body weight) the same day as ligation of renal lymphatics resulted in creatinine, paraaminohippurate and urea clearances which were considerably lower than the clearances in sham ligated dogs receiving the same dose of uranyl acetate. Clearances in dogs with renal lymphatic ligation alone were slightly lower than sham ligated dogs in agreement with results in a previous study (6). The clearances approached the control levels in 6 to 12 weeks *(Fig. 2)*. When injection of uranyl acetate was delayed until 2 weeks after the operation, there was minimal effect of the nephrotoxin and no distinct or consistent differences in the clearances in the ligated or sham-operated dogs.

### *Electrolyte and water clearance*

When uranyl acetate was administered the same day as renal lymphatic ligation or sham

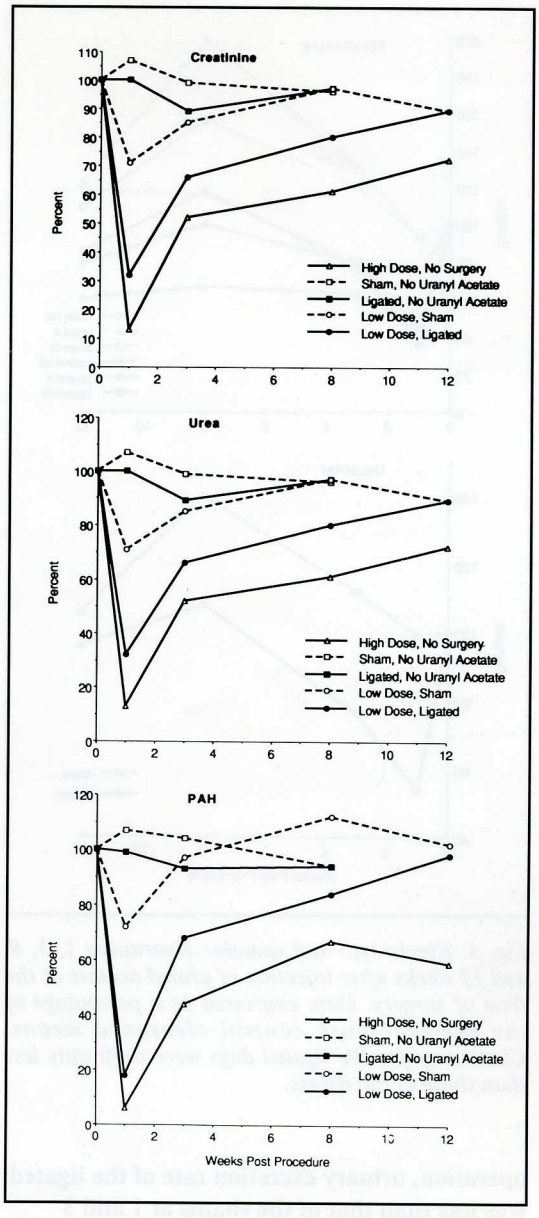


*Fig. 2. Creatinine, urea and PAH clearance at 1, 3, 8 and 12 weeks after injection of uranyl acetate and the day of surgery (0). Data expressed as a percentage of pre-op, pre-injection control clearances. The control clearances means are 100%. Ligation of renal lymphatics produced a response to a small dose of uranyl acetate (0.028-0.05mg/kg body weight) similar to the response to a large dose (0.2-1.0mg/kg) without lymphatic ligation. Ligation of renal lymphatics without uranyl acetate was associated with only a slight decrease in the clearances (6).*

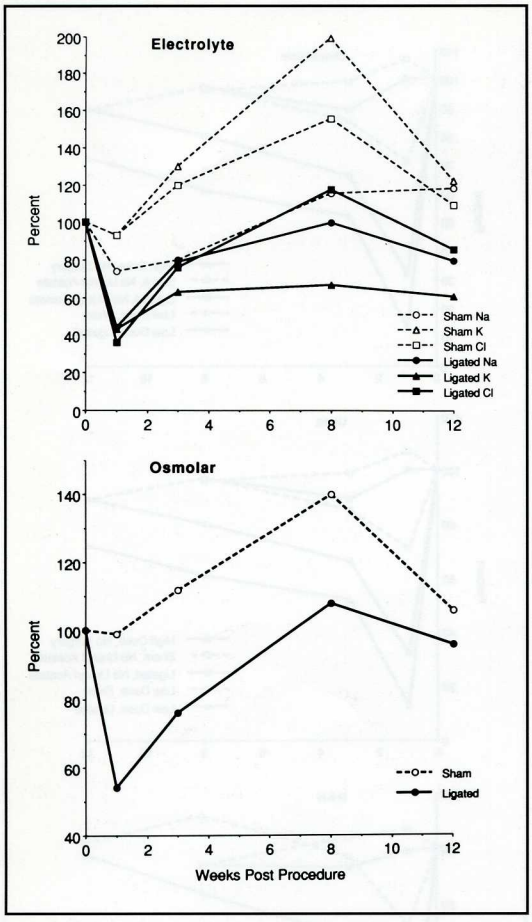


*Fig. 3. Electrolyte and osmolar clearances 1, 3, 8, and 12 weeks after injection of uranyl acetate at the time of surgery. Data expressed as a percentage of the pre-procedure control clearance means. Clearances for the ligated dogs were uniformly less than those of the shams.*

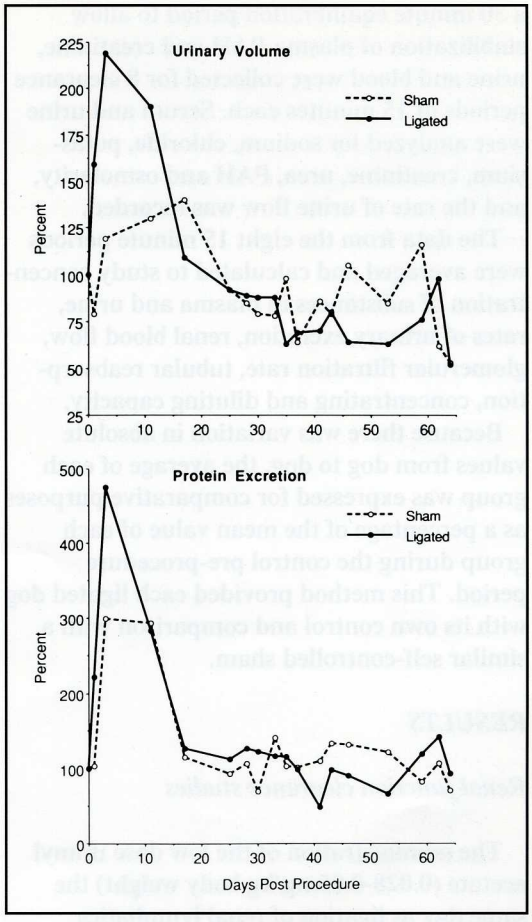


*Fig. 4. Average daily urinary volume and protein excretion as a percentage of the pre-op, pre-injection average daily excretion. The pre-procedure daily means are 100%. Although for the first three weeks protein excretion in both the ligated and sham was elevated, protein excretion in the ligated was greater than that in the sham.*

operation, urinary excretion rate of the ligated was less than that of the shams at 1 and 3 weeks and 2 and 3 months after injection and operation. Sodium, chloride, potassium and osmolar clearances were calculated to be lower for the ligated than for the sham *(Fig. 3)*. The electrolyte and osmolar clearance increased in the shams.

*Filtration fraction*

The filtration fraction was considerably greater in the ligated group for the first 8 weeks.

*Recovery of infused sodium, chloride and water*

Although the results were variable, generally volume and sodium excretion during and after the clearance periods were lower in the ligated compared with the sham.

*DAILY STUDIES*

*Urinary volume*

Although elevated in both groups, urinary excretion volume of dogs with ligated renal lymphatics was greater than that of sham ligated for approximately 3 weeks following the administration of uranyl acetate the same day as the operation. For the remainder of the 3 months of observation, the excretion rate in the two groups was essentially the same *(Fig. 4)*.

*Protein*

For approximately two and one half weeks the urinary protein was markedly elevated in both groups *(Fig. 4)*.

*Creatinine and urea*

Daily clearance of both creatinine and urea was distinctly lower in the ligated group than in the sham group; however, plasma levels were not elevated *(Fig. 5)*.

*Electrolytes*

Excluding the clearance days and the day following, the excretion of both sodium and chloride were decreased in both groups and generally slightly lower in the ligated than in the shams but the significance of the difference is questionable *(Fig. 6)*. Plasma levels were slightly higher in the ligated dogs.

*DISCUSSION*

Although the results suggest that a decrease in glomerular filtration rate (GFR) is related to decreased renal blood flow, they may also be explained as due to increased pressure in Bowman's space. Some reports indicate that tubular secretion is impaired faster than GFR (15). Using injection of India ink into the capsular and hilar renal lymphatics, both Bell *et al* (16) and we have demonstrated that lymphatics encompass the glomerulus and possibly participate in uptake of excess fluid and large molecules from within or around the capsule *(Fig. 7)*. If these lymphatics are impaired because of inflammation or obstruction, the pressure within the Bowman space may rise and impede glomerular filtration. Lymphatics also encircle the tubules and if these are non-functioning, tubular reabsorption may be compromised. It has also been demonstrated that in some animals with obstructed lymphatics, there are several glomerular and tubular histological abnormalities. In other studies (6,7), these derangements correlate with renal function, but we have not been able to arrive at any conclusion from the histologic examinations performed in this study.

The greater filtration fraction in the ligated group for the first 8 weeks suggests that decreased renal blood flow is responsible for the decrease in urea and creatinine clearance following renal lymphatic ligation. A decrease in PAH clearance in conjunction with decreased urea and creatinine clearance also suggest that decreased renal blood flow is responsible for a decreased GFR. Although variable, the generally lowered urinary volume and sodium excretion in the ligated compared with the sham group suggests that interrupted lymphatic function in conjunction with uranyl acetate alter the ability of the kidneys to respond to water and salt loads for up to 3 months. The return of clearances (PAH, creatinine, urea) toward control values in 6 to 12 weeks is compatible with regeneration and restored function of the obstructed, transected lymphatics. Permanent effects of ligation are not expected since it has been previously reported that alternate routes of lymph flow such as lymphatic-venous communications develop at varying times in the first few weeks after lymphatic ligation (7,13,14). Marked elevation in urinary protein in both groups signifies that uranyl acetate with or without ligation of renal lymphatics causes enough renal injury to promote proteinuria.

Lilienfeld *et al* demonstrated transient changes in renal function, blood pressure, intravenous urogram and renal angiogram after ligation of the renal lymphatics (4). Schreiner *et al* cited that uranium produced proximal tubular necrosis, deposition of hyaline and with large doses in rats central

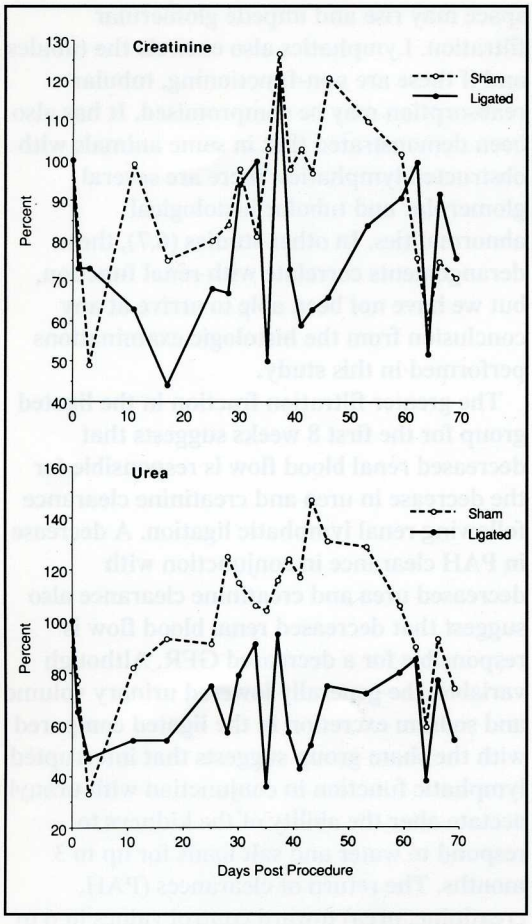


*Fig. 5. The average daily creatinine and urea clearances after injection of uranyl acetate and surgery (time 0) as a percentage of the pre-procedure control means. The rise of daily clearances of both creatinine and urea in the sham indicates an increase in GFR and a possible decrease in tubular reabsorption. Both clearances were lower in the ligated than in the sham.*

lobular lesions in some glomeruli. These findings were similar to some of our observations in both ligated and shams. Since the renal tissue was obtained three months after the procedures, and since the dose of uranium salt was much smaller in our studies, we could not interpret their significance.

Intravenous doses of uranyl nitrate in humans produces albuminuria, cylindruria and azotemia. With large doses, sodium resorption decreases along with potassium

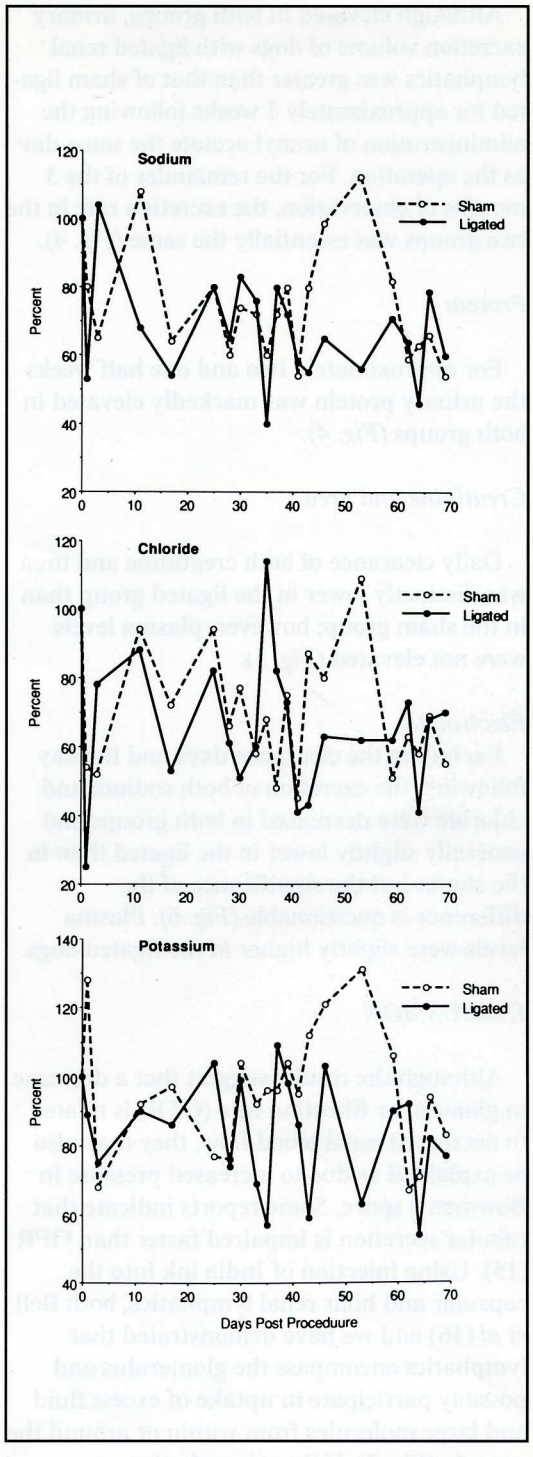


*Fig. 6. The average daily clearances of sodium, potassium and chloride as a percentage of the pre-procedure control means.*

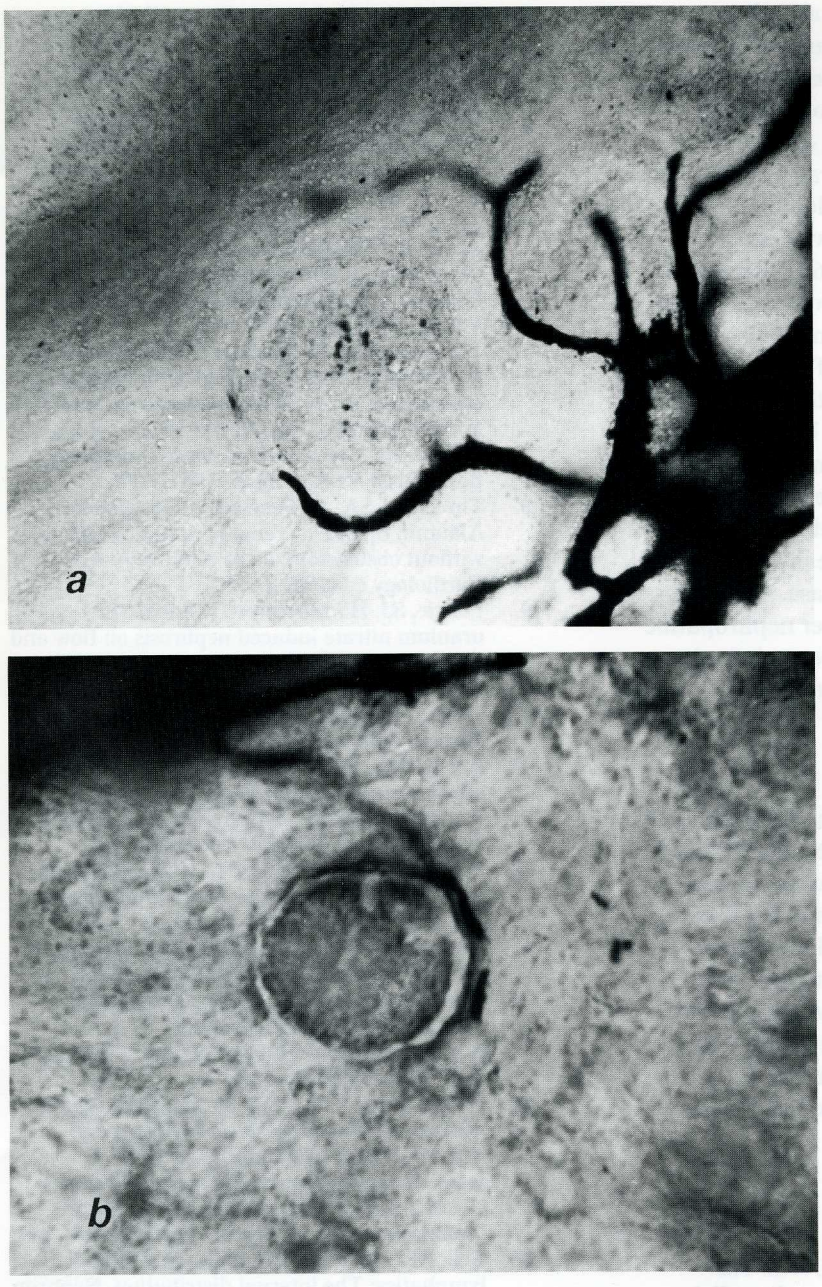


*Fig. 7a and b. Lymphatics filled with India ink after injection of the renal capsular lymphatics. The lymphatics appear to surround the glomerulus.*

secretion. The clearance of urea and creatinine does not fall (17). Whereas clearance decreases in our studies with large doses of uranyl acetate, it does not with small doses unless also accompanied by ligation of renal lymphatics. The fact that electrolyte, osmolar and urea clearances increase after uranyl acetate in the sham-ligated dogs suggests an interference with tubular reabsorption or increased glomerular filtration. The mechanism by which obstruction of the renal lymphatics modified this finding and produces lower electrolyte, osmolar and urea clearance is unclear. The results, however, support the

concept that multiple factors are often necessary to produce significant alteration in measurement of functional elements. Our studies fail to demonstrate, however, the development of chronic renal disease or nephrotic syndrome. Regeneration of ligated renal lymphatics and/or development of alternate routes of lymph flow occurs in otherwise normal animals (4,6,7). Because of this ability, it is difficult to produce an experimental counterpart of permanent disordered lymphatic uptake and flow. If lymphatic uptake or flow are permanently impaired by a condition which scleroses the lymph vessels, such as infection and/or inflammation, then functional changes seen in the first few weeks after ligation and administration of a nephrotoxin could possibly be prolonged and progress to chronic renal disease. Along these lines, further studies with other nephropathic agents such as aminoglycosides or infection to produce nephritis are warranted.

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