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The Flow Rate and Macromolecule Content of Hilar Lymph from the Rabbit's Kidney under Conditions of Renal Venous Pressure Elevation and Restriction of Renal Function – Studies on the Origin of Renal Lymph.*

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Summary

The aim of this investigation was to ascertain whether renal lymph can be correctly thought of as mixed product consisting of protein-free fluid reabsorbed from the renal tubules together with plasma.

By progressively compressing the terminal segment of the renal vein the hydrostatic pressure within it was raised to over 40 mmHg. The glomerular filtration rate (GFR) and the absolute amount of fluid reabsorbed fell simultaneously to 20% of their initial values. Lymph flow in a renal hilar lymphatic rose proportionately to renal vein pressure by a factor of up to 10. The concentrations of protein and polyvinylpyrrolidone (PVP, MW 110,000) in the renal lymph remained unaffected at levels of 76 ± 13% and 56 ± 21% of their respective plasma concentrations. The intrarenal ^{131}I-albumin pool also remained unchanged at 19.7 ± 4.3 ml plasma/100 g kidney. It is concluded that renal lymph is formed mainly by a filtration process from plasma. The results provide evidence against any admixture of protein-free reabsorbed fluid with the renal lymph.

Introduction

As long ago as 1931 *Drinker* et al. (5) postulated that renal lymph was derived from two sources: the protein-free reabsorbate from the renal tubules and a protein-containing plasma filtrate originating from the peritubular capillaries. If this mixture hypothesis is correct it would be expected that experimental modification directed at one or other of these sources would cause changes in the protein concentration of the lymph. The aim of the present study was to put this to the test. Depending on species and technique, the protein concentration of renal hilar lymph ranges from 30-70% of the plasma protein

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concentration (13; 16; 23; 29; 31; 34). In earlier investigations *Vogel* et al. (35) found some evidence in favour of the mixture hypothesis. When tubular reabsorption was partially blocked by giving furosemide lymph flow diminished and there was an increase in the lymph concentration of a macromolecular test substance (polyvinylpyrrolidone (PVP); MW 110,000), which can reach the interstitial tissues only via the peritubular capillaries. However, there was no corresponding increase in the protein concentration of the lymph.

In the present investigation the hydrostatic pressure in the peritubular capillaries was raised with the aim of increasing the inflow of fluid from the plasma into the interstitial tissue. If the mixture hypothesis were correct the increased admixture of protein-rich capillary filtrate with the interstitial fluid should raise the protein concentration of the lymph.

The present investigation was therefore designed to study the effect of raising renal vein pressure on the flow rate of renal hilar lymph and its protein and PVP (MW 110,000) concentrations. So as to collect adequate information regarding intrarenal physiology under raised venous pressure, measurements were also made of the albumin distribution volume in the kidney and of inulin, PAH and water clearances.

Material and Methods

The investigations were carried out on 32 crossbred rabbits of both sexes. They were kept in individual hutches, some of them out of doors, and were given a diet consisting solely of Altromin K® and water ad libitum for at least a week beforehand. They were starved for 16 hours before the beginning of the tests. Under pentobarbitone (Nembutal®) anaesthesia (40 mg/kg i.v.) and mild mannitol diuresis (25 mg mannitol/min · kg at a mean infusion rate of 0.5 ml/min) the abdomen was opened and the following surgical procedures were carried out at the hilum of the kidney, the technique being similar to that used in previous work (8):

1. Cannulation of a hilar lymphatic.
2. Exposure of the renal vein.
3. Introduction of a polyethylene tube (diameter 0.4x0.95 mm) through a puncture in the caudal vein opposite the opening of the left renal vein. The tube was then pushed deep into the left renal vein.
4. Application of a screw clamp to the renal vein close to its opening into the vena cava.
5. Exposure of the left ureter and cannulation with a PVC tube.

During the experiment, body temperature was kept constant by a heating pad which served as a support for the animal. Pressure in the renal vein was measured by means of a Statham recorder. Fifteen minutes before the beginning of the first lymph collection period a single intravenous infusion of PVP (MW 110,000) was slowly administered (400 mg/kg body weight in 20% solution). Lymph and urine were collected in two to five periods each of approximately 30 minutes duration. The blood levels were determined at the mid-point of each collection period. Plasma and lymph PVP levels were measured by the method of *Levy* and *Fergus* (17).

Renal function was evaluated by giving inulin and PAH by continuous infusion (1.2 ml/hour) and measuring the clearances. Urine flow rate was also determined. During the first clearance period the screw clamp was wide open, but in subsequent clearance periods the outflow resistance in the renal vein was progressively increased by gradually closing the screw clamp. In some cases the renal vein was totally occluded. Any experiments in which

there was a substantial diminution of PAH or inulin clearance during the first period were discarded because of the probability that the dissection had interfered with normal renal function.

To ascertain the albumin distribution volume, 16 animals were given 5 μCi of ^{131}I-albumin (human)* by injection into the ear vein before the termination of the experiment. Ten minutes later the hilar vessels of the left kidney were clamped and ligated close to organ, which was then removed and weighed. A blood specimen was taken at the same time. The quantity of ^{131}I-albumin in the kidney and in 1 ml of serum was determined by an established method (18) and the results were used to calculate the ^{131}I-albumin distribution volume, as existing 600 seconds after administration of the test substance.

Results

a) *The influence of renal vein constriction on the concentrations of albumin and PVP in renal lymph and on lymph flow and albumin distribution volume in the kidney*

The concentrations of albumin and PVP (MW 110,000) at various renal vein pressures were measured during 84 clearance periods in 32 rabbits. Figures 1c and 1d show the protein and PVP concentration in renal lymph, expressed as quotients (R) of the corresponding plasma concentrations, at various hydrostatic pressures in the renal vein. They show that when venous pressure was raised from 8 to 40 mmHg, the concentrations of PVP and plasma protein in the renal lymph remained unchanged. The stated regressions do not differ from 0 ($p > 0.5$). Even in individual animals there were no definite differences in these concentrations or quotients at different pressures. For PVP (MW 110,000) R was 0.56 ± 0.21 and for protein 0.76 ± 0.13.

Pressure rise in the renal vein produced a proportionate increase in lymph flow. Figure 1b shows that as venous pressure increased lymph flow rose to as much as 10 times its initial values. The results suggest that there is a linear relationship between the two quantities.

In earlier investigations (8; 35) it was found that the renal blood vessels and an easily accessible extravascular albumin distribution volume were filled approximately 10 minutes after intravenous administration of ^{131}I-albumin to rabbits, the amount of albumin being equal to that contained in 18.5 ± 1.2 ml of plasma. Approximately 22% of this protein must be situated in the interstitial space. The aim of the present investigations was to ascertain whether this albumin pool is increased by raising the pressure in the renal vein. Figure 1a reproduces the results obtained in 18 experiments. There was no perceptible influence. In these experiments the albumin pool was unaffected by pressure changes in the renal vein and was equivalent to the quantity of albumin contained in 19.7 ± 4.3 ml of plasma. It is thus approximately similar to the quantity found in previous experiments on rabbits, the hilar vessels of which had not been dissected (vertical grey column in Fig. 1a), in keeping with the results obtained by *Vogel* et al. (35).

b) *The influence of renal vein constriction on inulin and PAH clearances and on fractional reabsorption of water*

Constriction of the renal vein is followed by a fall in inulin clearance, the fall being roughly proportional to the change in PAH clearance; the filtration fraction amounts to 14.5 ± 5%.

*^{131}I-albumin (human) was kindly supplied by Farbwerke Hoechst AG, Frankfurt-Hoechst.

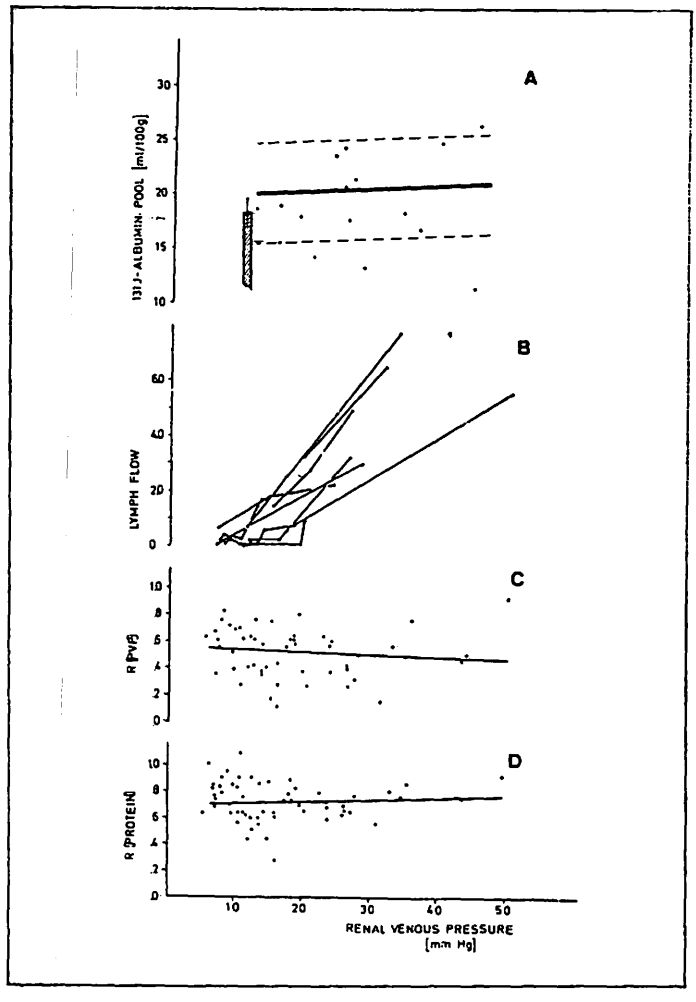

Fig. 1 Effect of increasing hydrostatic pressure in the rabbit's renal vein on
a) ^{131}I-albumin distribution volume in the kidney, 10 minutes after administration of the test substance. ($y = 0.035\ x + 19.5$; $S = 4.3$; $N = 16$)
b) lymph flow per minute in a single hilar lymphatic,
c) PVP (MW 110,000) concentrations in hilar lymph expressed as quotients (R) of the corresponding plasma concentrations. ($y = -0.002\ x + 0.56$; $s = 0.21$; $N = 57$)
d) the ratio (R) of protein concentrations in hilar lymph and peripheral blood. ($y = 0.002\ x + 0.7$; $s = 0.13$; $N = 58$)

Figures 2a and b show that even minimal constriction is enough to cause striking reduction in these renal function indices. If the constriction is enough to produce an interstitial pressure of over 40 mmHg they may fall to below 20% of normal.

Repeated measurements of mean systemic arterial pressure during our experiments showed that it remained approximately the same over the entire period of observation, amounting to approximately 90 mmHg. Pressure in the abdominal vein was also substantially constant, remaining between 4 and 8 mmHg.

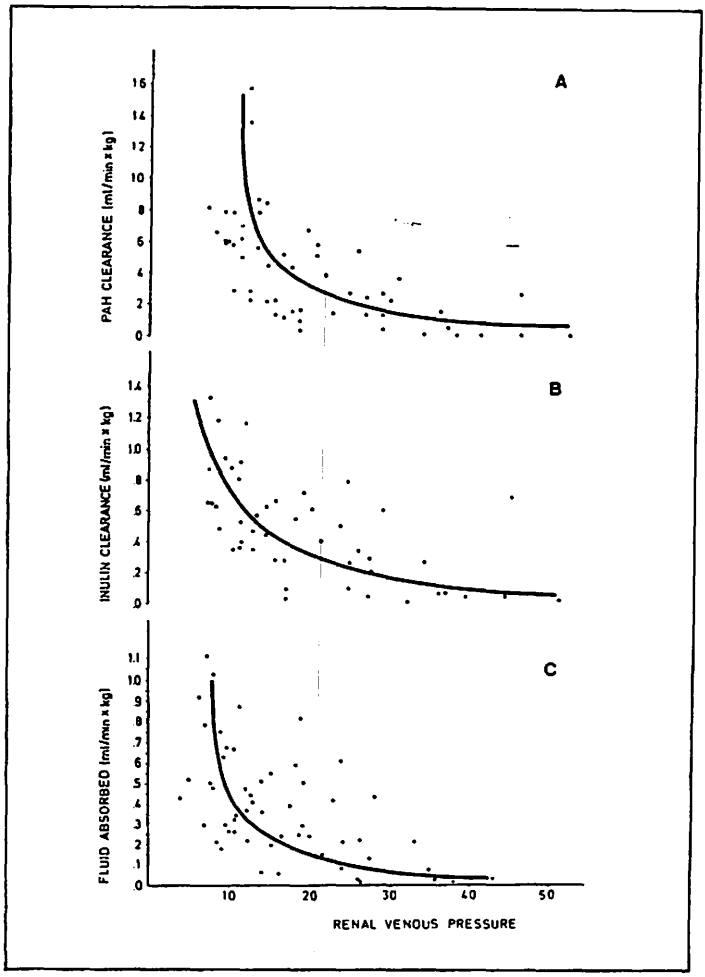

Fig. 2 (Drawn by eye). Effect of renal vein pressure on: a) PAH clearance, b) inulin clearance, c) volume of fluid reabsorbed.

From the inulin clearances and the urine flow rates it is possible to calculate the absolute and fractional water reabsorption in the kidney at various renal vein pressures. Figure 2c shows the influence of venous constriction on the absolute quantity of fluid reabsorbed by the tubules. This corresponds to the stream of protein-free fluid which flows through the interstitium of the kidney. When the renal veins are unconstricted the amount reabsorbed is approximately 0.5 ml/min by one kidney per kg rabbit (Fig. 2c). Raising the venous pressure to 40 mmHg causes the absolute quantity of fluid reabsorbed to fall below 10-20% of the initial value.

Discussion

Various authors (8; 19; 21; 22; 28; 30; 32; 37) have suggested that the interstitial fluid

of the kidney contains plasma protein in a state of rapid exchange with the plasma proteins in the blood.

Investigations in rats and rabbits (8; 35) indicate that the amount of plasma protein exuding every minute out of thepertubular cpillaries into the renal interstitium is equvalent to the amount contained in 2.5 ml serum per 100 g kidney. In golden hamsters, however, *Wilde* et al. (37) and *Pinter* et al. (26) were unable to confirm that quantity is of this order. At the same time some protein-free reabsorbate enters the interstitium.

In the present experiments this amounted to 0.5 ± 0.025 ml/min · kg rabbit in the left kidney where no venous constriction was applied. Calculated in terms of 100 g renal tissue this gives a reabsorbed fluid volume of 10-12 ml/min. A concentration equilibrium for protein became established between the protein-containing exudate and the protein-free reabsorbate, the level at which it was set depending on the relative amounts of these two inputs and the filtration properties of the capillaries and lymphatics through which the macromolecules are removed. In these experiments raising the pressure in the renal vein depressed GFR to approximately 20% of its intial value and reduced the quantity of protein-free reabsorbate flowing through the interstitial tissue from 10-12 to approximately 1-2 ml/100 g kidney. At the same time lymph flow rose to as much as 10 times its initial rate, a change which undoubtedly augmented the quantity of protein passing into the interstitial tissues. From both these facts a rise in interstitial fluid protein concentration would have been expected. However, analyses of lymph did not confirm this. The lymph concentrations of protein and PVP (MW 110,000) – a molecule which behaves similarly – remained constant at 76 ± 13% and 56 ± 21% of their respective plasma concentrations. This constancy in the face of rising venous pressure is at variance with the results obtained in similar experiments on muscle (4; 10). It is evidently a special feature of the kidney, as may be seen from the results of other workers (1; 12; 16; 33). Another respect in which the kidney differs from other tissues is that the albumin distribution volume within the organ is independent of increasing peritubular pressure (10). All these facts cast doubt on the mixture hypothesis. Moreover, they cannot be explained away by the observations of *Falchuk* et al. (6), who noted a fall in protein concentration in the immediately proximal peritubular capillaries when venous pressure was raised.

Garlick and *Renkin* (10) and *Renkin* (27) have analysed the conditions governing the passage of macromolecules through capillary walls and have criticized the conclusions of *Pappenheimer* et al. (24; 25), *Grotte* et al. (11), *Mayerson* et al. (16) and others. They believe that the endothelial pores in muscle preparations from dogs or cats have a radius of approximately 30 Å, and that small macromolecules (MW < 20,000) pass through mainly by convection while larger ones (MW 40,000-100,000) penetrate mainly by diffusion. In the case of capillaries with fenestrated endothelium, as for example in the liver, the intestinal tract and the peritubular capillaries of the kidney, larger pore diameters must be postulated. The data of *Mayerson* et al. (16) for the liver and intestinal tract and of *LeBrie* (15) and *Gärtner* et al. (8) for the peritubular capillaries of the kidney indicate that the mean pore diameter is so large that molecules of the size of albumin can easily pass through by convection. The existence of these special permeability conditions in the postglomerular capillaries of the kidney is substantiated by the present work. It suggests that macromolecules find their way into renal hilar lymph by means of a filtration process.

In view of these experimental results there must be some doubt whether the filtration process which determines the protein concentration of the lymph actually takes place

at the peritubular capillary endothelium. The peculiar features of lymphatic drainage in the kidney (14) and of its interstitial ultrastructure (2) also suggest that this filtration can be carried out by interstitial structures or by the walls of lymph capillaries. The present findings therefore do not permit any definite statement to the effect that the protein concentrations in hilar lymph and interstitial fluid are identical.

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Influence of Renal Fluid Dynamics on Renal Lymph Pressure, Flow and Composition[1]

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Summary

The effects of decreased RBF on renal lymph formation and TP were investigated during experimentally maintained IRVP. It was found that this procedure is effective in maintaining TP and L_CP at or above control even in the absence of RBF. While lymph PAH and creatinine concentrations were unchanged under these circumstances, lymph protein concentration was increased. It is concluded that IRVP is a major factor determining lymph and tissue pressure and that increases above control may be due to a combination of physical factors and tissue ischemia.

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