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THE LYMPHATICS OF THE KIDNEY AND THE FORMATION OF RENAL LYMPH

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SUMMARY

1. The anatomy of the renal lymphatics and the flow and composition of renal lymph have been investigated in sheep. Lymphatic capillaries were demonstrated in the cortex and cortico-medullary areas of the sheep's kidney but not within the medulla. The lymph formed in the sheep's kidney drains from the hilum through several small lymphatics; there is no lymphatic drainage from the renal capsule.

2. Lymph flow from the sheep's kidney varied from 0·5 to 3·0 ml./hr. The concentrations of electrolytes and urea in renal lymph were found to be similar to lymph from other regions of the body, but the concentration of endogenous creatinine was lower. The average concentration of protein in renal lymph was 43% of the plasma levels; there was a significantly higher proportion of albumin in renal lymph than in plasma. When labelled albumin was injected intravenously, the specific activities of the plasma and renal lymph albumin equilibrated in about 2 hr.

3. When [^{14}C]inulin, [^{125}I]hippuran or [^{14}C]creatinine were infused intravenously radioactivity appeared rapidly in the renal lymph. When steady-state levels were reached in the circulating plasma, the renal lymph/jugular vein plasma ratios for [^{14}C]inulin and [^{14}C]creatinine were 0·82 while for [^{125}I]hippuran the ratio was 0·34. It was considered that the concentration of these substances in renal lymph was similar to the concentration in renal vein plasma.

4. The concentration of renin enzyme was on the average about 8 times higher in renal lymph than in jugular vein or renal vein plasma or in lymph from the hind limbs.

5. Renal lymph appeared to be formed principally, if not entirely, in the renal cortex and appeared to be a modified filtrate derived from post-

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glomerular blood capillaries. If any contribution to renal lymph does come from the medulla, it must be small in relation to the volume of lymph formed in the cortex.

INTRODUCTION

The precise distribution of lymphatics within the kidney and the origin and composition of renal lymph is poorly understood. Lymphatics are difficult to demonstrate in the kidney parenchyma by conventional injection procedures and long-term, quantitative collections of renal lymph under physiological conditions do not seem to have been accomplished.

Most collections of renal lymph have been from dogs. The multiplicity of the capsular and hilar lymphatics in this and other species makes the quantitative collection of renal lymph impossible. To overcome this problem, various indirect experimental approaches have been used to estimate the extent of renal lymph production. Schmidt & Hayman (1930) measured the thoracic duct lymph flow in dogs almost totally eviscerated, and took this as a measure of renal lymph production. Bull & Metaxas (1962) stripped the kidney from its surrounding tissue attachments and after placing the kidney in a basin they collected the fluid oozing from the cut lymphatics. O'Morchoe & O'Morchoe (1968) calculated renal lymph flow rates in the dog by measuring the rate of thoracic duct flow before and after ligating the renal artery. The fall in the thoracic duct flow that followed this procedure was considered to be the rate of renal lymph production. Small volumes of lymph have been collected acutely from single renal lymphatics and the total lymph production in the kidney calculated by multiplying the volume collected by a factor representing the average number of lymphatics present (Sugarman, Friedman, Barrett & Addis, 1942; Mayerson, 1963). Attempts have also been made to calculate renal lymph production indirectly by measuring various parameters such as glomerular filtration rate, clearance ratios, and lymph protein concentrations (Földi, 1963).

There are objections to all of these indirect methods of assessing renal lymph production, obvious from the fact that the published results of the papers show lymph flow rates for dogs of standard weight, varying over more than a 100-fold range. The present paper records the results of experiments in sheep in which the distribution of the lymphatics of the kidney has been studied and quantitative collections of renal lymph made in conscious animals under physiological conditions for periods of weeks. The origin of renal lymph has been investigated and its composition has been compared with the plasma and with lymph from other sources.

METHODS

Animals

Merino and Dorset Horn–Merino ewes, 6 months to 2 years of age and weighing between 22 and 40 kg, were used for all experiments. They were kept in metabolism cages and fed lucerne chaff and grain oats daily with free access to water. Water and food were withheld for 24 hr before surgery.

Surgical preparations

Most operations were performed on the left kidney as it is more conveniently placed than the right, and has a longer, more mobile pedicle. The kidney was approached retroperitoneally through an incision made immediately below the lateral processes of the lumbar vertebrae and parallel to the spine. One or two lymph nodes are found at the origin of the renal artery where it leaves the aorta; these are the regional lymph nodes draining the kidney. The afferent lymphatics of these nodes, usually three to five in number, were followed back to the renal hilum and the largest and most accessible vessel was cannulated with a polyethylene tube (usually 0·59 mm i.d., 0·9 mm o.d.). The other lymphatics were tied off. The cannula was anchored to the surrounding tissues with stay sutures, and brought out through the psoas major muscle above the line of the incision. In some experiments more than one lymphatic vessel was cannulated. The efferent lymphatic leaving the popliteal lymph node was also cannulated in some experiments (Hall & Morris, 1962).

The ureter was cannulated with a polyvinyl tube (i.d. 1·4 mm, o.d. 1·9 mm) and the end of the tube passed into the ureter so as to lie about 3–4 mm from the renal pelvis. It was tied in position with three sutures and brought outside through the caudal end of the incision.

A polyethylene cannula (i.d. 1·0 mm, o.d. 1·2 mm) was introduced into the renal vein in some experiments, either through an adjacent venous branch or through the wall of the renal vein itself and tied in place. Systemic intravenous infusions were given through a polyethylene cannula inserted into the jugular vein. Blood samples were taken from the jugular vein opposite to the one into which material was being infused.

All experiments were done after the ewes had recovered from surgery and were eating normally, usually 2–3 days after the operation. Lymph was collected for analysis only if it flowed continuously for a period of at least a week.

Labelled substances

[^{125}I]Hippuran, creatinine-1-[^{14}C] hydrochloride (the Radiochemical Centre, Amersham), [^{14}C]carboxyl inulin (New England Nuclear Corporation, Boston) were diluted in sterile isotonic saline (0·9 g NaCl/100 ml.) and infused at a constant rate with a peristaltic pump (Sigmamotor). When necessary, priming intravenous injections of the labelled material were given. In experiments designed to study the equilibration of albumin between plasma and renal lymph, single injections of [^{131}I]human serum albumin (the Radiochemical Centre, Amersham) were made into the jugular vein, and lymph and venous blood samples collected over the next 6 hr.

Analytical methods

The radioactivity in samples of plasma and lymph was measured with a windowless gas-flow proportional counter (Tracerlab Automatic Flow Counter) or with a Liquid Scintillation Spectrometer (Beckman Model L 5200). For scintillation counting,

plasma and lymph samples were prepared by first adding a solution of trichloracetic acid (10 g/100 ml.) to precipitate the proteins. Na^+, K^+, Mg^{2+} and Ca^{2+} concentrations were measured with an atomic absorption spectrophotometer (Techtron AA-4 model) after preparing the samples according to the methods described by Willis (1960*a–c*).

Urea was estimated by the method of Roijers & Tas (1964) and protein by Kjeldahl digestion and titration. Endogenous creatinine was measured by the method of Edwards & Whyte (1958).

Renin was assayed in samples of lymph and jugular venous plasma by the method described by Skinner (1967). Sheep renin substrate was prepared from blood taken from a nephrectomized virgin ewe.

Histological techniques

Demonstration of lymphatics. Lymphatics were visualized with Berlin blue (Edward Gurr, 2 g/100 ml. distilled water) or India ink (Gunther Wagner, Pelikan) diluted with an equal volume of NaCl solution (0·9 g/100 ml.) injected through a 30-gauge needle inserted into the subcapsular tissue. Sections of tissue in which lymphatics had been visualized were fixed in formol-saline, dehydrated and cleared in equal parts of methyl salicylate and benzyl benzoate (Spalteholz, 1914).

Electron microscopy. Small pieces of tissue were taken from kidneys of sheep anaesthetized with pentothal sodium after the lymphatics had been identified with Berlin blue. The tissue was placed immediately into either glutaraldehyde (1·25 ml./100 ml.) in 50 % (v/v) Tyrode solution for 2–4 hr at 4° C or osmium tetroxide (1 g/100 ml.) in 50 % (v/v) Tyrode solution for 2 hr at 4° C. Subsequent post-fixation, preparation and examination of the tissues was as described previously (Pedersen & Morris, 1970).

Light microscopy. Tissue for light microscopy was fixed in formol-saline and embedded in paraffin. Sections were stained with haematoxylin and eosin, Mallory's Trichrome, van Gieson's or the periodic acid–Schiff reaction.

RESULTS

The lymphatic drainage of the sheep's kidney

The lymphatic drainage of the sheep's kidney occurs by way of two to five hilar lymphatics, 0·5–1·5 mm in diameter, which run to the renal lymph node. These lymphatics are found usually beside or between the renal artery and vein. The renal lymph node, which is located near to the origin of the renal artery from the aorta, is often haemal in character and there are usually several small haemolymph nodes nearby. The efferent lymphatics from the renal lymph node join with the lumbar trunk which runs cranially towards the cisterna chyli.

No lymphatics were found running from the renal capsule. The capsule of the sheep's kidney is thin and inelastic and has no blood supply from the perirenal tissues. This arrangement meant that all the lymph produced in the kidney drained from it by way of the hilar lymphatics.

Lymphatics within the kidney. When a solution of T-1824-albumin was injected into the cortex of the kidney, the renal hilar lymphatics turned blue almost at once. This demonstrated that there was a rapid drainage

of material from the renal cortical tissues. Lymphatics in the cortex were difficult to inject in normal kidneys but were easy to demonstrate if the ureter had been ligated or partially obstructed some time previously.

Within the kidney the lymphatics formed a profuse network beneath the capsule and around the interlobular vessels, the arcuate vessels of the cortico-medullary junction and the inter-lobar vessels (Pl. 1*A–C*). It was not possible to inject any lymphatics in the renal medulla, although collecting lymphatics were found running with the major blood vessels as they passed to the hilum. These lymphatics were mostly within the adventitia of the arteries and veins. Lymphatics could also be seen beneath the epithelium lining the renal pelvis and the ureter; they were particularly prevalent in these situations in hydronephrotic kidneys (Pl. 1*D*).

The injected vessels were identified as authentic lymphatics by electron microscopy. Their ultrastructure was similar to lymphatics in other tissues. The endothelium of many of the larger lymphatic vessels had numerous folds and projections so that in certain sections there were spaces in the walls of the vessels. Many of these spaces were associated with endothelial junctions, suggesting that, along their length, adjacent cells were widely separated in places. Gaps and open junctions were also present in many of the renal lymphatic capillaries.

The rate of production and composition of renal lymph. Irrespective of whether one or several renal lymphatics were cannulated, the total amount of lymph collected hourly from the kidney was the same. It was concluded that there were extensive interconnexions between lymphatics in the kidney before they reached the hilum and thus quantitative collections could be made by cannulating one of the hilar lymphatics and tying off the rest.

Renal lymph often contained red blood cells and when this was so, the lymphatics appeared sanguineous when they were first exposed. This phenomenon was more frequent in sheep over 2 yr of age and it was thought to be the reason why the renal lymph node was often haemal in character. Many of the red cells passed through the node and were found in the efferent renal lymph and in the thoracic duct. In young animals (under 2 yr of age) red cells were virtually absent from the lymph and free-flowing cannulations could be prepared which would function for periods of several weeks.

Flow rates from different animals varied between 0·5 and 3·0 ml./hr but were constant over periods of days in the one individual. There seemed to be little correlation between the rate of lymph flow and the volume of urine produced, as when ewes were deprived of water for 3–4 days they produced very little urine, while the rate of lymph flow from the kidney remained unchanged.

TABLE 1. The concentration of protein, urea, creatinine and electrolytes in the jugular vein plasma and renal lymph of normal ewes. Mean results are shown together with their standard errors. Some figures are also given for leg lymph and urine

	Total protein (g/100 ml.)	Urea (mg/100 ml.)	Creatinine (mg/100 ml.)	Sodium (m-equiv/l.)	Potassium (m-equiv/l.)	Magnesium (m-equiv/l.)	Calcium (mg/100 ml.)
Renal lymph	2·80 ± 0·07	47·5 ± 1·0	0·78 ± 0·06	148·8 ± 1·3	4·0 ± 0·04	1·2 ± 0·02	8·1 ± 0·09
Plasma	6·47 ± 0·07	48·2 ± 1·2	0·88 ± 0·04	144·8 ± 0·9	4·3 ± 0·03	1·5 ± 0·02	9·4 ± 0·07
Renal lymph/ plasma ratio	0·43	0·99	0·88	1·03	0·93	0·81	0·85
No. of sheep	10	13	9	13	13	12	14
Leg lymph	1·28 ± 0·04	48·3 ± 1·0	0·87 ± 0·03	—	—	—	—
Urine				1·7	139·5	24·0	2·5

The composition of renal lymph compared with plasma is given in Table 1.

The average protein content of renal lymph was about 43% of the plasma level. The A/G ratio for renal lymph was 1·36 whereas for plasma it was 0·69. The concentrations of sodium and urea in renal lymph were similar to those in plasma, while the concentrations of calcium and magnesium were slightly lower in accordance with the Gibbs-Donnan equilibrium. In these respects renal lymph resembled leg lymph very closely. Endogenous creatinine concentrations however were significantly lower in renal lymph than in jugular vein plasma or in leg lymph (Table 1).

The partitioning of clearance substances between plasma and renal lymph. [^{14}C]Inulin, [^{14}C]creatinine and [^{125}I]hippuran were infused intravenously into sheep with renal lymphatic cannulations over periods of 4–5 hr until steady-state concentrations were achieved in the jugular vein plasma. Lymph and plasma samples were collected every 30 min during this time and analysed for radioactivity. In some experiments with [^{14}C]inulin the popliteal lymph was also collected and analysed.

The labelled substances appeared rapidly in the renal lymph but at equilibrium the levels in renal lymph were always significantly lower than the levels in the jugular vein plasma or in the lymph coming from the hind limbs. In the case of inulin and creatinine the renal lymph/jugular vein plasma ratios averaged 0·82 when steady-state concentrations were reached. The leg lymph/jugular vein ratios for [^{14}C]inulin were unity. However, when blood samples were taken from the renal vein it was found that the level of labelled inulin in renal vein plasma was 80% of the level in the jugular vein plasma and equal to the level in renal lymph (Text-fig. 1).

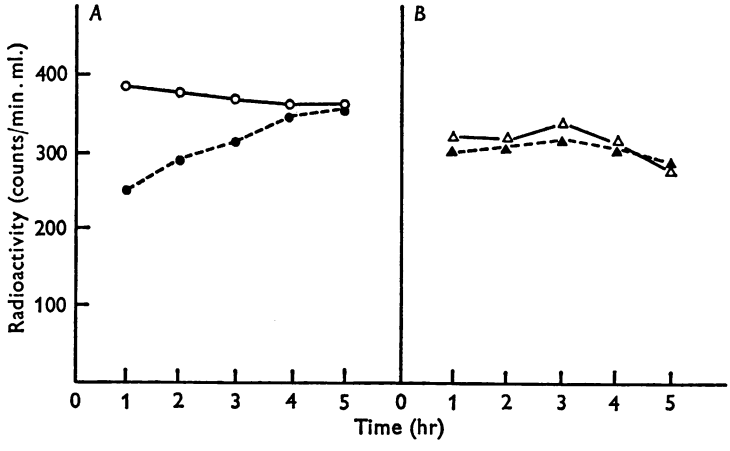
The average renal lymph/plasma ratio for [^{125}I]hippuran at equilibrium was 0·34, the range of four experiments being 0·31–0·38. The extraction ratio for hippuran was not determined in these experiments but data obtained in other species have shown that about 70% of hippuran is cleared by the kidney (Meschan, Schmid, Watts & Witcofski, 1963) and it was thought that the content of hippuran in renal lymph also reflected the concentration of hippuran in renal venous plasma.

The results of experiments in which the partitioning of inulin, creatinine and hippuran between jugular vein plasma, renal lymph and leg lymph was studied are given in Table 2.

The exchange of albumin between the plasma and renal lymph. The exchange of albumin between the circulating plasma and the renal lymph was studied in six sheep by injecting 10–20 μc of [^{131}I]-labelled human serum albumin intravenously and following the changes in the specific activity of the plasma and lymph.

Equilibration occurred rapidly between the plasma and lymph, so that by 2 hr after injection the specific activity of the albumin in the renal lymph exceeded that of the plasma. From this time on the plasma and lymph specific activities remained similar (Text-fig. 2).

The amount of albumin in the interstitial pool of the kidney drained by the renal lymphatics was calculated (Morris, 1956). The average weight of the kidneys in these experiments was 61 g and the interstitial pool was



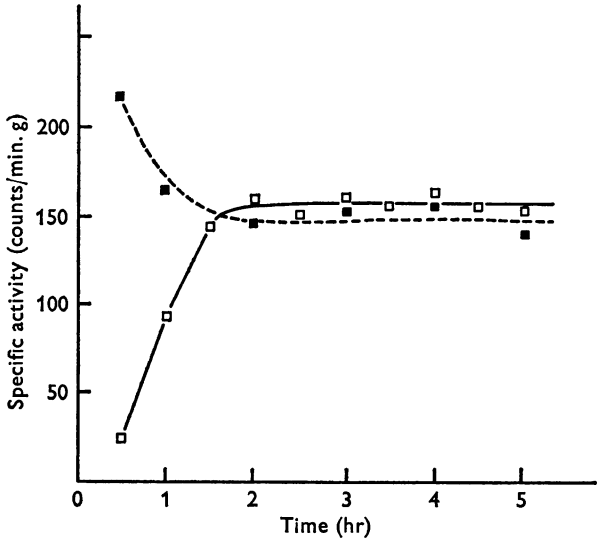
Text-fig. 1. The concentrations of radioactivity (counts/min.ml.) in the jugular vein plasma and the popliteal lymph (*A*) and in the renal vein plasma and the renal lymph (*B*) of a sheep infused intravenously with [^{14}C]inulin for a period of 5 hr. ○——○, jugular vein plasma; ● - - ●, popliteal lymph; △——△, renal vein plasma; ▲ - - ▲, renal lymph.

TABLE 2. The renal lymph/jugular vein plasma ratios (R.L/P) for [^{125}I]hippuran, [^{14}C]inulin and [^{14}C]creatinine infused intravenously into normal ewes for periods of 4–5 hr. During the [^{14}C]inulin infusions, leg lymph was collected as well as renal lymph in two experiments; the leg lymph/jugular vein plasma ratios (L.L/P) measured at the end of the infusion period are also shown. Lymph and plasma samples were collected at half-hourly intervals after equilibration had occurred, and were assayed for radioactivity. The mean results are given for six separate measurements made during each infusion

	[^{14}C]inulin		[^{14}C]creatinine		[^{14}C]hippuran	
Expt. no.	R.L/P ratio	L.L/P ratio	Expt. no.	R.L/P ratio	Expt. no.	R.L/P ratio
1	0·83 ± 0·01	—	5	0·87 ± 0·02	7	0·38 ± 0·01
2	0·81 ± 0·02	0·96	6	0·77 ± 0·01	8	0·32 ± 0·02
3	0·86 ± 0·03	1·03	—	—	9	0·35 ± 0·01
4	0·77 ± 0·01	—	—	—	10	0·31 ± 0·03
Over-all mean	0·82	—	—	0·82	—	0·34

estimated to contain, on the average, 17 mg of albumin and to be about 2 % of the weight of the organ.

The content of renin in renal lymph. Renal lymph and jugular vein plasma samples from ten different animals were assayed for their renin content. At first, renal venous plasma samples were assayed concurrently with jugular venous samples. It was found, however, that the content of renin in the jugular and renal vein plasma was the same within the limits of



Text-fig. 2. The specific activities (count/g) of the plasma and renal lymph albumin of a sheep given an intravenous injection of [^{131}I]human serum albumin. ■ – – ■ jugular vein; □——□ renal lymph.

accuracy of the bio-assay; subsequently only jugular vein samples were assayed with renal lymph. Samples of leg lymph were also assayed, but their content of renin was scarcely measurable.

The concentration of renin in renal lymph varied in individual animals from 0·8 to 20·0 renin units/ml. with a mean of 10·8 units/ml. In all animals, however, the concentration of renin in the renal lymph was significantly higher than in the plasma. The mean renal lymph/plasma concentration ratio for renin was 7·7.

DISCUSSION

An important feature of the lymphatic drainage of the sheep's kidney is that there are no capsular lymphatics and lymph drains from the kidney exclusively by way of vessels leaving the hilum. This enables complete collection of renal lymph in this species, and by using chronic cannulation

techniques on conscious animals it is possible to make valid estimates of renal lymph production. This anatomical arrangement in the sheep offers particular advantages for the study of problems associated with renal transplantation (Pedersen & Morris, 1970).

The flow rates of renal lymph measured for the sheep have been low when compared with some of the rates calculated or inferred in other species. In the dog, where many capsular lymphatics are present, renal lymph flow rates have been estimated to be as high as 150 ml./hr (Bull & Metaxas, 1962). O'Morchoe & O'Morchoe (1968) came to the conclusion that 15–33% of the thoracic duct lymph flow in the dog came from the kidneys. These flow rates certainly do not occur in the sheep and, in view of the indirect methods used to obtain them and the almost certain haemodynamic changes which would follow manoeuvres such as clamping the renal artery and evisceration, these results probably do not give any true indication of the physiological rates of renal lymph production.

Contrary to what has been suggested, it does not seem that the lymphatic drainage of the kidney plays any unique role in regulating the fluid balance of the organ, apart from the general functions that lymphatics subserve in other tissues. In this regard the special role of the kidney in excretion, and the formation of urine with high concentrations of urea and electrolytes are not reflected in the volume of renal lymph produced or in its composition.

Sugarman *et al.* (1942) and LeBrie & Mayerson (1959) found that the concentrations of urea, sodium and chloride in the renal lymph of dogs were significantly higher than in the plasma. It has been shown that the countercurrent region of the renal medulla contains a high concentration of sodium and urea (Ullrich & Jarausch, 1956; Schmidt-Nielsen & O'Dell, 1959) and Mayerson (1963) inferred that renal lymph formation occurred within the medulla. Keyl, Scott, Dabney, Haddy, Harvey, Bell & Ginn (1965) did not confirm these results in the dog and the present experiments show no differences in the concentrations of sodium and urea in renal lymph and plasma in the sheep. This evidence lends no support to the proposition that a significant amount of renal lymph is formed in the medulla.

The results obtained with clearance studies were similar to those of Keyl *et al.* (1965) in the dog. The concentrations of inulin, creatinine and hippuran in renal lymph indicated that this lymph is probably formed throughout the cortex of the kidney and that it is in equilibrium with renal venous plasma.

Pressor activity, thought to be due to renin, has been found in samples of renal lymph collected acutely from anaesthetized dogs (Lever & Peart, 1962). In all sheep examined renal lymph contained significantly higher

concentrations of renin than renal or jugular vein plasma. In terms of the relative rates of renal blood and lymph flow, the amount of renin transported in the renal lymph is small compared with the blood. The high lymph concentrations probably reflect a high local concentration of renin in the tissue fluid of that region of the kidney where the enzyme is produced, and this may have important implications in feed-back control of synthesis and release of the enzyme or in the regulation of local metabolic events. The high concentration of renin in the lymph also provides further evidence that the cortex is the main site of renal lymph production. Peart (1959) showed in rabbits and Blair-West, Coghlan, Denton, Scoggins, Wintour & Wright (1967) showed in sheep that there was a concentration gradient of renin from the outer cortex to the cortico-medullary junction. No renin was found in the medulla.

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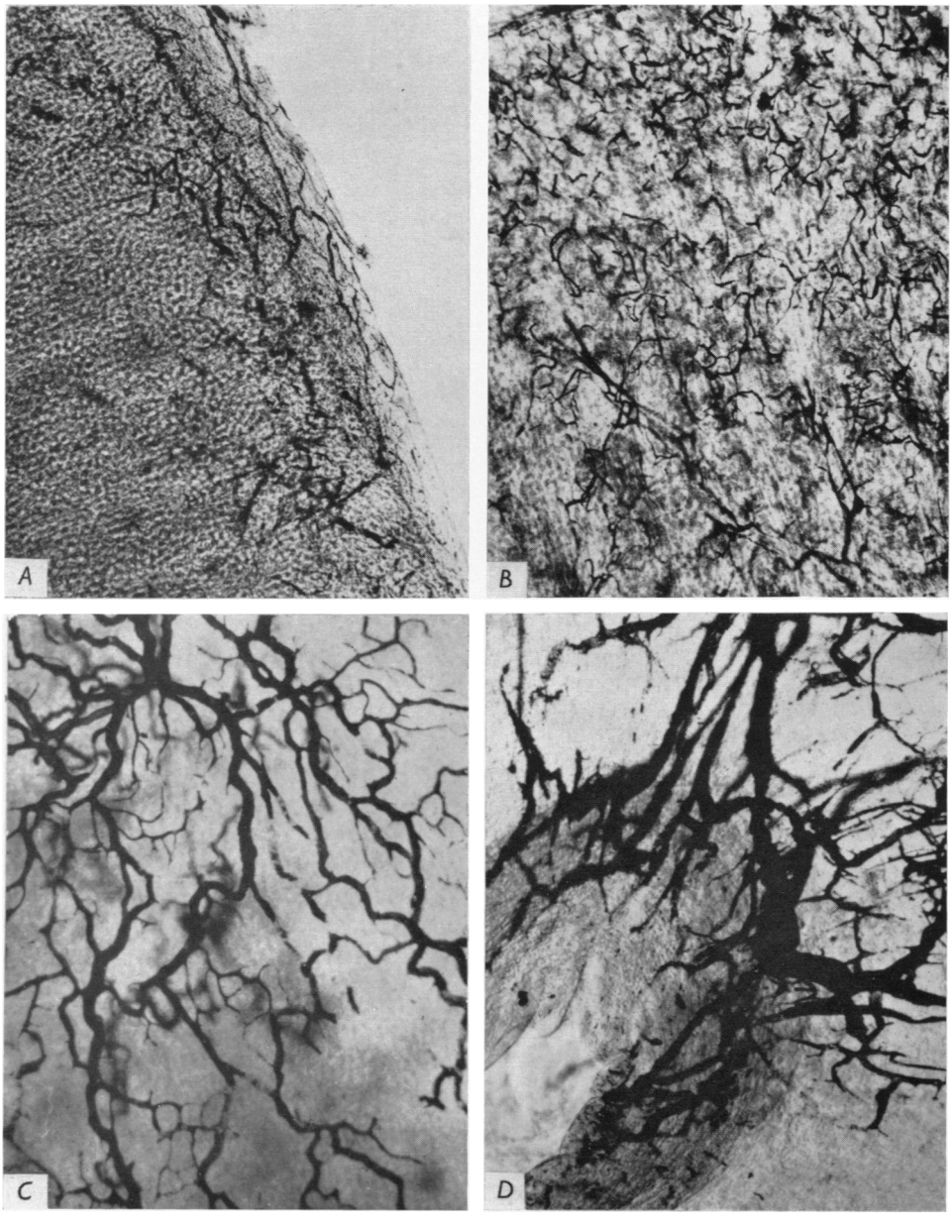
EXPLANATION OF PLATE

A and *B*. Cleared thick sections (approx. 3 mm) of the cortex of a sheep's kidney, showing networks of lymphatics injected with India ink. *A*, × 27; *B*, × 13.

C. A cleared mount of renal subcapsular lymphatics viewed *en face*. India-ink injection into a kidney in which the ureter had been ligated previously. × 9·3.

D. Lymphatics in the renal pelvis injected with Berlin blue. Cleared mount showing connexions with the lymphatics of the ureter. × 8.



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