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THE NATURE OF EXPERIMENTAL SECOND-SET KIDNEY TRANSPLANT REJECTION

2. THE MIMICKING OF THE HAEMODYNAMIC UPSET BY PHARMACOLOGICAL AND OTHER MEANS

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SUMMARY.—The second-set kidney transplant reaction involves in its early stages vasoconstriction of the outer cortex. This is a phenomenon occurring widely in medical and surgical conditions and is precipitated by stimuli of pharmacological, nervous, immunological and ischaemic origins. Various stimuli elicit varying degrees of outer cortical vasoconstriction and although the renal vascular response usually involves all segments, the outer cortical segments can remain in spasm when the more proximal segments have been pharmacologically vasodilated. Vasoconstriction in the outer cortex leads to impaired renal function and explains the transient anti-diuretic action of drugs such as nor-adrenaline, Hypertensin-Ciba (Ciba), ergot preparations, vasopressin and Priscol (Ciba) which can mimic the vasoconstriction induced by warm and cold ischaemia and the second-set reaction. The marked vasoconstricting ability of the vessels of the outer cortex, as part of the renal response to various stimuli, is seen as an unfortunate by-product of an evolutionary mechanism designed to cater for constant adjustments in the handling of salt and water. The factors mediating the second-set reaction remain obscure since vasodilators, α-adrenergic blockers and smooth muscle paralysers are all ineffective in combating the vasoconstriction.

THE second-set kidney transplant reaction is one which affects primarily the blood vessels at some stage after transplantation and usually within 24 hr. Arteriographic monitoring indicated that general afferent renal vasoconstriction was the cause of the onset of acute renal failure (Dempster, 1953*a*, *b*). Recent experiments have indicated that the earliest detectable change occurred in the outer cortex involving exclusion of this area from renal perfusion (Pineo, Regoeczi and Dempster, 1970). This was not, in our view, a new phenomenon for in previous experiments dealing with the effects of adrenaline (Dempster and Joekes, 1955), Priscol (Dempster, 1954*a*), Renin (Dempster, 1970*c*), cold ischaemia (Dempster, Kountz and Jovanovic, 1964; Aboul-Enein, Paccione, Todd, Shikata, Dempster and Kountz, 1965), and first-set kidney rejection (Dempster, 1953*a*, 1970*a*) similar renal haemodynamic effects have been described.

Acute renal failure due to functional spasm of the afferent arteriolès and glomerular capillaries will not necessarily be revealed in histological sections. Indeed, this was the case in assessing impaired glomerular function in first-set kidney transplant rejection with respect to the absence of obvious morphological changes in the glomeruli (Dempster, 1953*a*). Arteriography provides a means of

assessing the immediate cause of acute renal failure in observing the distribution of blood flow in the kidney. The problem still remains, however, as to what tissue damage is primary and what is secondary and what the nature of the stimuli are which evoke outer cortical vasoconstriction. It was considered to be of some importance for a greater understanding of the second-set reaction if a formal analysis was made of changes in outer cortical perfusion due to various stimuli such as cold and warm ischaemia, vasopressor drugs and increased renal venular pressure.

MATERIALS AND METHODS

Surgical procedures.—Greyhound bitches of about 25 kg. were used. The left kidney was autotransplanted to the iliac vessels in the manner previously described (Dempster, 1954*b*). The right kidney acted as the control. Arteriograms and nephrograms were taken after injections of various drugs and various procedures. The general effect on the kidneys and blood pressure was recorded.

(1) Transplantation of second-set kidneys

As previously described (Pineo *et al.*, 1970).

(2) Cold ischaemia for 20 hr.

As previously described (Aboul-Enein *et al.*, 1965).

In addition, the effects were observed of various drugs on the altered renal haemodynamics immediately after preservation and transplantation in a series of 6 dogs. These drugs were Phenoxybenzamine (Dibenylene (Smith, Kline and French) in doses of 50 mg. i.v.), β-receptor blocker Propanolol (Inderal (I.C.I.) i.v. 5 mg.), Frusemide (Lasix (Hoechst) in doses of 20 mg. i.v.) and Papaverine (in doses of 25 mg. i.v.). The effects of these drugs were recorded by arteriography and renal function studies involving measurement of urine volume and Phenol Red (PSP) excretion.

(3) Warm ischaemia for 2 hr.

A light bull-dog clamp was placed on the left renal artery with the minimum of mobilization. The right kidney was kept as control. Six dogs were subjected to this procedure. About 15 min. after restoring the circulation an arteriogram was made and 10 min. later a nephrogram. Phenoxybenzamine and Frusemide were injected to determine whether the arteriographic appearances could be reversed. About 2 hr later total blood flow was measured by severing the renal vein and collecting for 15–30 sec.

(4) Increased venular pressure in allotransplanted kidneys.

As previously described (Dempster, 1970*a*).

(5) Intrarenal haemodynamic upsets.

As previously described (Dempster, 1954*a*, 1969).

Modification of preserving kidneys for 20 hr as previously described by Aboul-Enein et al., 1965.—After flushing with warm plasma-rheomacrodex-procaine solution, a cold solution of similar constitution was flushed through with a large syringe. No clamps were left on the artery or vein during preservation. Prior to transplantation, the right kidney was removed in order to study the immediate function of a kidney preserved at 4° for 20 hr. About 30 min. after opening the new circulation 20 mg. Frusemide were injected i.v. and the effect recorded by measuring the rate of urine flow.

China Ink (Pelikan) injections of kidneys after preservation for 20 hr.—These kidneys were flushed out with the warm solution containing procaine followed by the cold solution and stored at 4° for 20 hr. After this period, the kidneys were removed, rewarmed and then injected with 5 ml. of China ink. The renal pedicle was clamped and the whole kidney was placed in formol-saline. Later the kidneys were sectioned in the longitudinal plane and photographed.

(b) Three kidneys were flushed out with the standard solutions minus procaine and stored for 20 hr. After storage the kidneys were rewarmed and were then treated as in Group I.

Injections of vasopressor drugs.—(a) Injections of adrenaline i.v.—previously described (Dempster and Joekes, 1955). (b) Injections of Priscol (tolazine hydrochloride). As described previously (Dempster, 1954*a*). (c) Angiotensin (Hypertensin-Ciba). As previously described (Dempster, 1970*b*). (d) Ergot preparations—Ergotamine tartrate (Femergin, Sandoz) and Ergometrine maleate (Syntometrine, Sandoz) in doses of 0·25 mg. i.v. (e) Injections of Infundin (Burroughs Wellcome) in doses of 1 unit i.v. (Dempster and Joekes, 1953).

(f) Mersalyl as a representative of mercurial diuretics, claimed to be vasoconstrictors (Hook, Blatt, Brody and Williamson, 1966). Two ml. Mersalyl (Evans Medical) were injected i.v. and 10 min. later the time of appearance and percentage excretion of PSP was measured. During a water diuresis Mersalyl was injected followed 10 min. later by 0·05 mg. Hypertensin. Arteriograms of kidneys were made 10 min. after an injection of Mersalyl.

Injection of α-adrenergic blocking drugs. Phenoxybenzamine.—(a) When a second-set reaction had been established. (b) When the vasoconstrictor effect of Hypertensin had been established or prior to its establishment. (c) After preserved kidneys had been transplanted. (d) After the release of circulation at the end of a period of 2 hr total warm ischaemia.

The effect was monitored in each case by arteriography.

Arteriograms and nephrograms.—With the kidney lying on a film (Ilford Industrial C) contrast medium (Thorotrast (Testagar) or sodium diatrizoate (Hypaque, Bayer) 65 per cent) was injected as previously demonstrated (Dempster, 1953*a*). Arteriography aimed to demonstrate the arterial filling phase at 0·1 sec. exposure at 75 kV and 100 mA and X-ray tube placed at a distance of 12 in.

Renal function studies were confined to assessing effective renal plasma flow by the phenol red test (Levinsky and Relman, 1963) and measuring urine volumes during diuresis and anti-diuresis.

Diuresis and anti-diuresis.—Six dogs were used and each experiment repeated on 3 occasions on each dog. Each dog was given one litre of water by stomach tube 30 min. prior to the start of each experiment. The experimental conditions were as follows: (a) At the height of a water diuresis 0·05 mg. Hypertensin was injected i.v. (b) At the height of a water diuresis 20 mg. Frusemide was injected i.v. (c) At the height of a water diuresis 0·05 mg. Hypertensin was injected i.v. followed immediately by 20 mg. Frusemide. (d) At the height of a water diuresis 0·05 mg. Hypertensin was injected i.v. followed 5 min. later by 20 mg. Frusemide. (e) At the height of a water diuresis 20 mg. Frusemide were injected followed 5 min. later by 0·05 mg. Hypertensin. (f) At the height of a water diuresis 0·25 mg. Ergotamine tartrate or 0·25 mg. Ergometrine maleate was injected i.v. followed 10 min. later by 10 mg. Frusemide. (g) At the height of a water diuresis Frusemide was injected followed 5 min. later by an i.v. injection of 1 unit of Infundin.

β-adrenergic blockade.—A similar series of experiments were carried out using β-adrenergic blockade with propanolol (Inderal, I.C.I.) in doses of 5 mg. i.v.). The dogs tolerated very well a dose of 5 mg. i.v. and no antidotes were required. The drug was injected over a period of 5 min.

Ganglion blockade by Pentolinium.—A series of experiments were performed to assess the effect of ganglion blockade on Infundin injected at the height of a water diuresis. Pentolinium tartrate 10 mg. (Ansolysen, May and Baker was given i.v. and 30 min. later 1 unit of Infundin was injected.

Histology.—Kidneys were fixed in 10 per cent formol saline within 5 min. of removal. Sections were cut at 5 μm. and routine stains used were Haemotoxylin and Eosin, Picro-Mallory and PAS.

RESULTS

The natural history of second-set kidneys

This has been described previously (Dempster, 1953*a*; Pineo *et al.*, 1970). Figs. 1, 2, and 3a illustrate the range of immediate reactions on opening up the new circulation as assessed by arteriography 30 min. later.

The effect of cold ischaemia for 20 hr has been described (Aboul-Enein et al., 1965)

Immediate oliguria or anuria after transplantation is due to temporary vasoconstriction of the outer cortex (Dempster, 1970*a*). Dibenylene was not found of value in releasing the post-transplant vasoconstriction. Provided oliguria was present 20 mg. i.v. of Frusemide increased the urine flow 3 fold for a few hr. Papaverine is of value in vasodilating the vessels as far as the interlobulars (Dempster, 1970*b*).

Fig. 4a, b illustrate the nature of renal perfusion in the immediate post-transplant phase. In spite of various drugs used to relax the outer cortical vessels some spasm remains. This will eventually relax and can be aided by an i.v. injection of Frusemide. Gross underperfusion which is usually associated with irrecoverable tubular damage does not respond to Frusemide.

China ink injections

Fig. 5 demonstrates the value of procaine in the flush-out solution. It aids outer cortical perfusion, but does not completely overcome all areas of outer cortical vasoconstriction. The functioning preserved autotransplants while not yet concentrating and acidifying normally at 3 weeks were able to maintain the animals in an apparently normal state of health.

Warm ischaemia for 2 hr

Fig. 6 demonstrates the type of arteriogram which consistently follows 2 hr of warm ischaemia. The total renal blood flow was reduced by 20–30 per cent for any given kidney weight. Such kidneys are immediately anuric on restoring the blood flow but a proportion can recover function several weeks later. Fig. 6a illustrates impaired renal perfusion and in some areas there is no outer cortical flow. The fact that a subnormal nephrogram can be associated with anuria is explained on the basis that the glomeruli of the inner cortex and to some extent the outer cortex, are able to filter the contrast material. The widespread damage to the tubules prevents adequate function. Phenoxybenzamine and Frusemide had no effect on outer cortical vasoconstriction as assessed by arteriography.

Increased venulae pressure in allotransplanted kidneys

Afferent vasoconstriction follows after renal vein pressure has been markedly raised (Dempster, 1970*a*). A distinction has to be made between a normal kidney with its extensive collateral venous drainage (Dempster, 1957) and a transplanted kidney with no immediate alternative venous drainage.

Vasopressor drugs

Adrenaline in doses of 80 μg., induces renal afferent vasoconstriction with momentary interference of outer cortical distribution (Dempster and Joekes, 1955). This may be precipitated by increased venular resistance since this has been described (Smith, 1951). The final momentary effect is an extensive afferent vasoconstriction with reduced renal volume and size (Fig. 7a, b). During this phase there is anuria which can be explained by the exclusion from perfusion of a proportion of the outer cortical glomeruli. Intravenous injections of adrenaline can be used to demonstrate by arteriography that in some cases of oliguria and anuria the resistance vessels are not maximally stimulated (Fig. 7d).

Priscol produces general afferent vasoconstriction affecting the whole of the arterial tree but initially particularly the outer cortical vessels (Fig. 8a, b). Arterial blood pressure rises about 20 mm. Hg. following the injection of 25 mg. i.v. Although total blood flow is reduced with transient exclusion of the outer cortex the overall distribution of flow returns quickly to normal and so urine production is drastically reduced only for a short period of about 5 min. Following such injected doses of Priscol there is a transient but marked anti-diuretic

EXPLANATION OF PLATES

FIG. 1.—(a) Renal arteriogram 30 min. after allotransplantation showing the extent of near normal perfusion in the arterial phase. The glomeruli in the cortex when perfused normally with contrast material render a snow storm effect obliterating any clear view of the interlobular arteries. The contrast material was Hypaque. White arrow points to an area from which a biopsy was removed. (b) Normal nephrogram taken 5–10 min. after the injection of Hypaque. This indicates that the nephrogram results from glomerular filtration of the contrast material. (c) The same kidney as illustrated in Fig. 1a some hr. after transplantation to a sensitized recipient and showing the early signs of disturbed cortical flow, *i.e.* outer cortical vasoconstriction. The kidney is now oliguric. (d) The nephrogram taken of the kidney illustrated in Fig. 1c. Although the kidney is severely oliguric there is a nephrogram of subnormal proportions due to the fact that some outer cortical glomeruli and most inner cortical glomeruli are still filtering.

FIG. 2.—(a) A slightly subnormal arteriogram of a second-set kidney with moderate functional capacity at 1 hr. after transplantation. The effective renal plasma flow, as measured by PSP excretion, was 15 per cent excretion in the first 15 min. (Normal for 1 kidney 20 per cent.) (b) An arteriogram of the kidney illustrated in Fig. 2a. A few hr later when signs of the second-set reaction were obvious. There is now gross underperfusion of the outer cortex in large areas of the kidney and anuria is established. (c) The subnormal nephrogram of the kidney illustrated in Fig. 2b taken 5–10 min. after the injection of Hypaque.

FIG. 3.—(a) The arteriogram of a transplanted kidney which succumbed immediately to a second-set reaction. If the afferent system is compared with the afferent systems in Fig. 1 and 2 it will be apparent that severe total afferent vasoconstriction has occurred. There is gross under-perfusion of the cortex and the pallisade effect of the visible interlobular arteries is now apparent and the normal snow-storm effect is absent. (b) The poor nephrogram of the kidney illustrated in Fig. 3a taken 5–10 min. after the injection of the contrast material. This illustrates that a poor nephrogram usually indicates subnormal outer cortical perfusion.

FIG. 4.—(a) The typical arteriographic pattern of a cold preserved kidney 30 min. after transplantation. There is marked underperfusion of the outer cortex in spite of the vasodilator effect of papaverine on the other segments of the renal arterial tree. The pallisading effect of the interlobular arteries can be clearly observed and this is diagnostic of underperfusion of the outer cortex. (b) The poor nephrogram of the kidney illustrated in Fig. 4a. The line running round the juxta-medullary zone is an artefact due to the globular nature of the kidney.

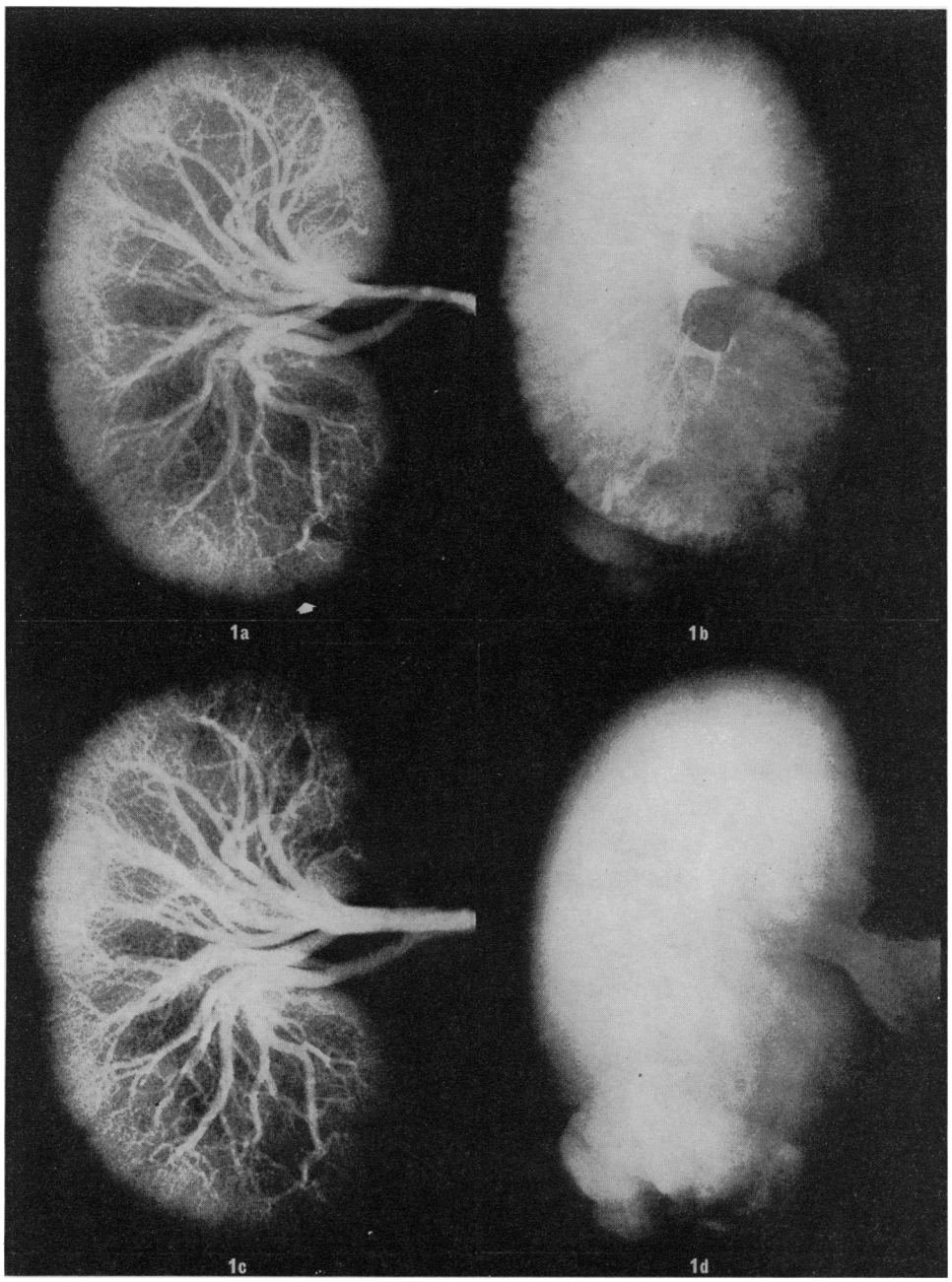
FIG. 5.—China ink injections of cold preserved kidneys immediately after the rewarming process. The kidney on the left was flushed out with plasma-rheomacrodex-heparin solution and the one on the right was flushed out with a similar solution but with procaine added. Procaine has a moderate action in relieving outer cortical vasoconstriction due to cold ischaemia and this results in more extensive perfusion of the outer cortex.

FIG. 6.—(a) The arteriographic pattern typical of the kidney subjected to 2 hr warm total ischaemia. Areas of moderate perfusion interspersed with areas of poor perfusion of the outer cortex add up to a net effect of anuria and severe cortical necrosis. (b) The poor nephrogram of the kidney illustrated in Fig. 6a taken 5–10 min. after the injection of contrast material.

FIG. 7.—(a) A normal renal arteriogram 24 hr after autotransplantation. The area of apparent non-filling at the pole is an artefact. (b) The same kidney 30 sec. after an i.v. injection of 80 μg. nor-adrenaline. Note that the kidney has contracted considerably, that there is total afferent vasoconstriction and zones of inadequate outer cortical perfusion. At this moment the kidney is anuric. (c) The arteriographic appearances of a kidney which has succumbed after a few hr to a second-set reaction. The kidney is anuric. (d) The same kidney as illustrated in Fig. 7c. Thirty sec after an i.v. injection of 80 μg. nor-adrenaline. It will be observed that the effect of the injected nor-adrenaline has proved that the outer cortical vasoconstriction in the kidney 7c. was not maximal although adequate in effecting anuria.

FIG. 8.—(a) The renal arteriographic pattern observed after an i.v. injection of 25 mg. Priscol. There is afferent vasoconstriction and a reduction of outer cortical filling involving all segments of the renal vascular tree. (b) A magnified view of an area of outer cortex in the kidney illustrated in Fig. 8a. This degree of reduction in outer cortical perfusion evokes an anti-diuresis lasting up to 2 hr.

FIG. 9.—(a) The renal arteriographic pattern developing within a few seconds of an i.v. injection of 0·05 mg. Hypertensin. Immediate anuria ensues. The kidney capsule becomes wrinkled as after an injection of nor-adrenaline. The kidney recovers perfusion in 10 min. but the anti-diuretic action may last for a further 50 min., suggesting stimulation of ADH. (b) Frusemide (20 mg. i.v.) has been injected into the recipient of the transplanted kidney. Five min. later Hypertensin (0·05 mg. i.v.) has been injected and an arteriogram taken 40 sec. later when already the vasopressor effect of Hypertensin is being antagonized. (c) The same kidney 4 min. later when practically full Frusemide renal vasodilatation and diuresis have been restored.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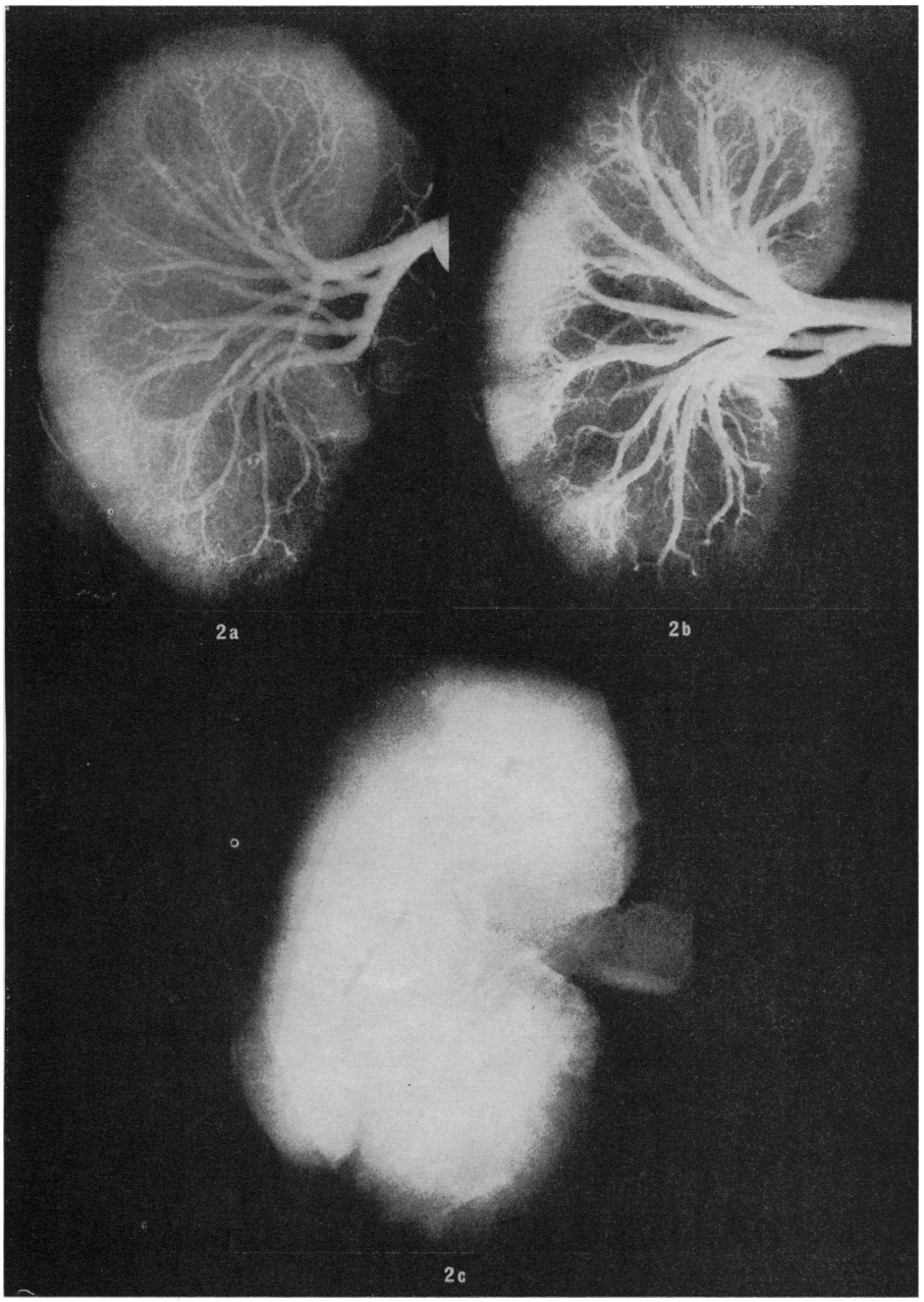
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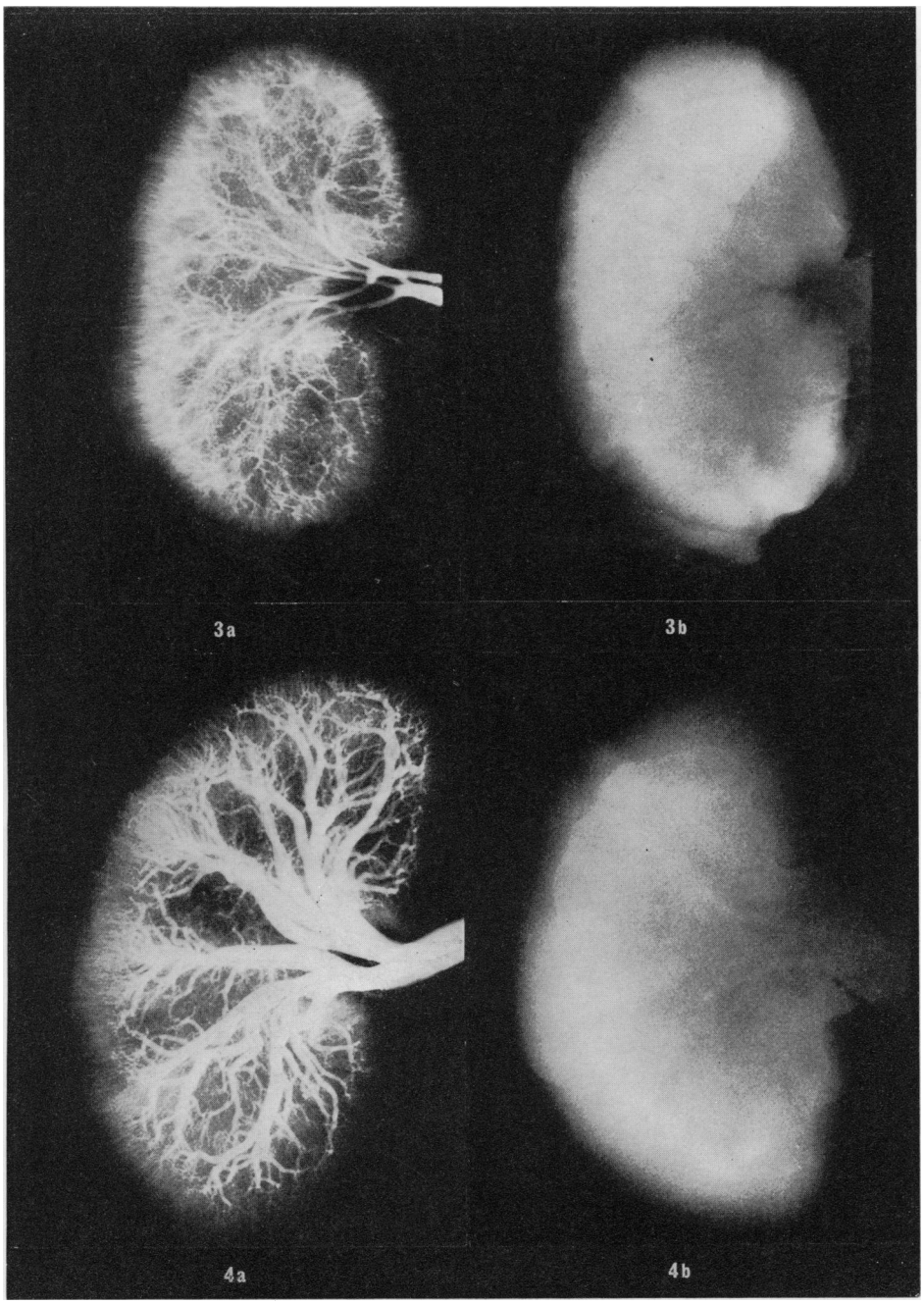
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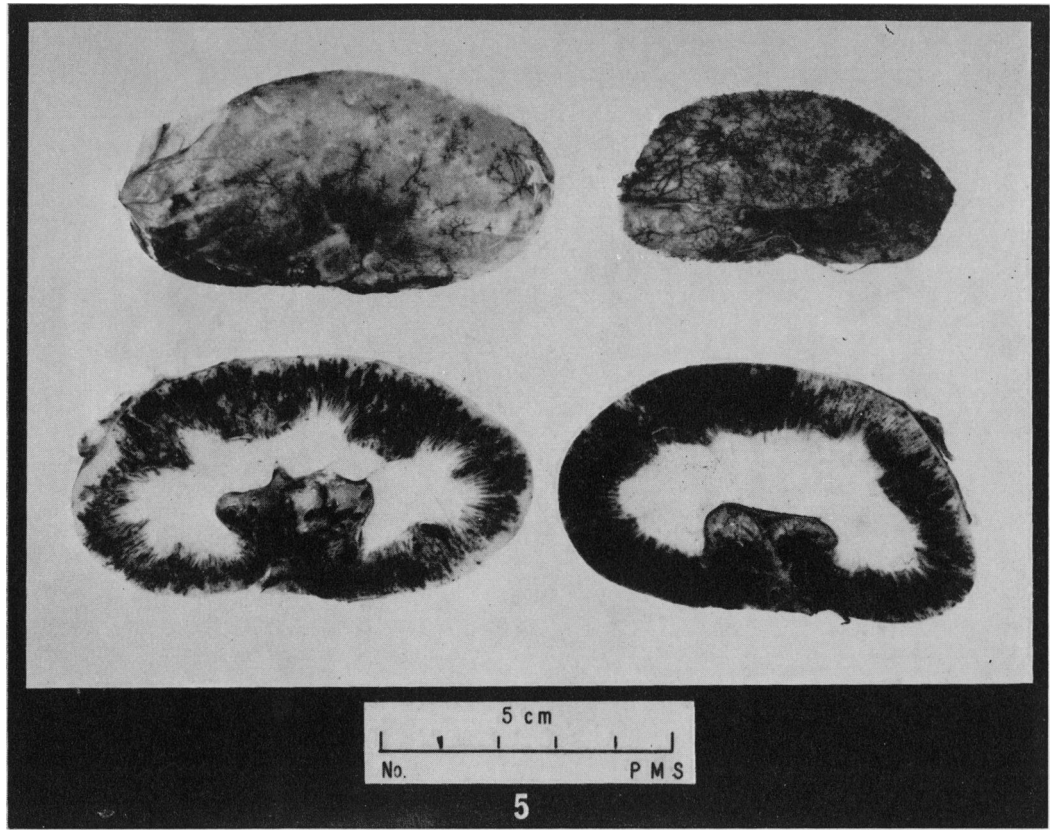

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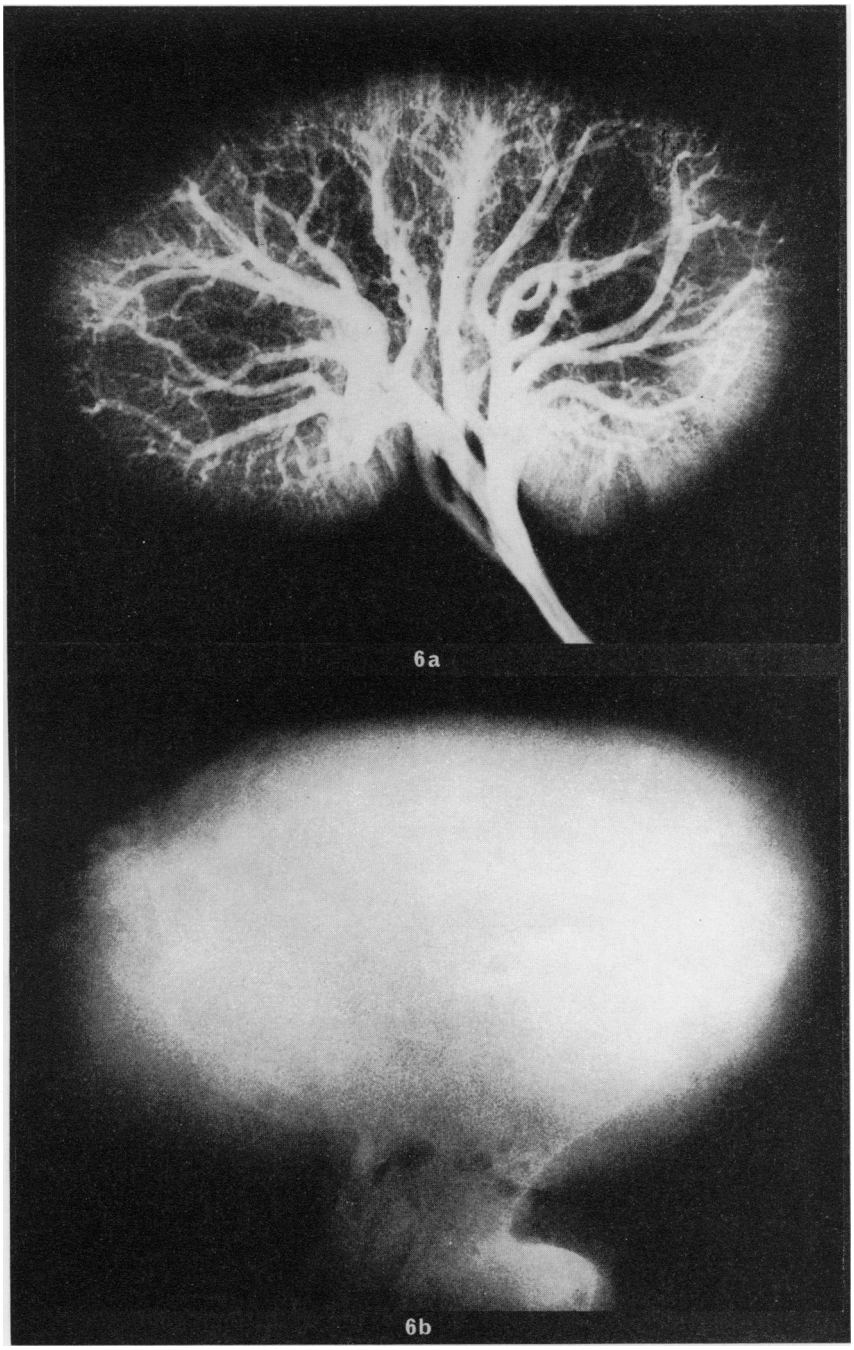
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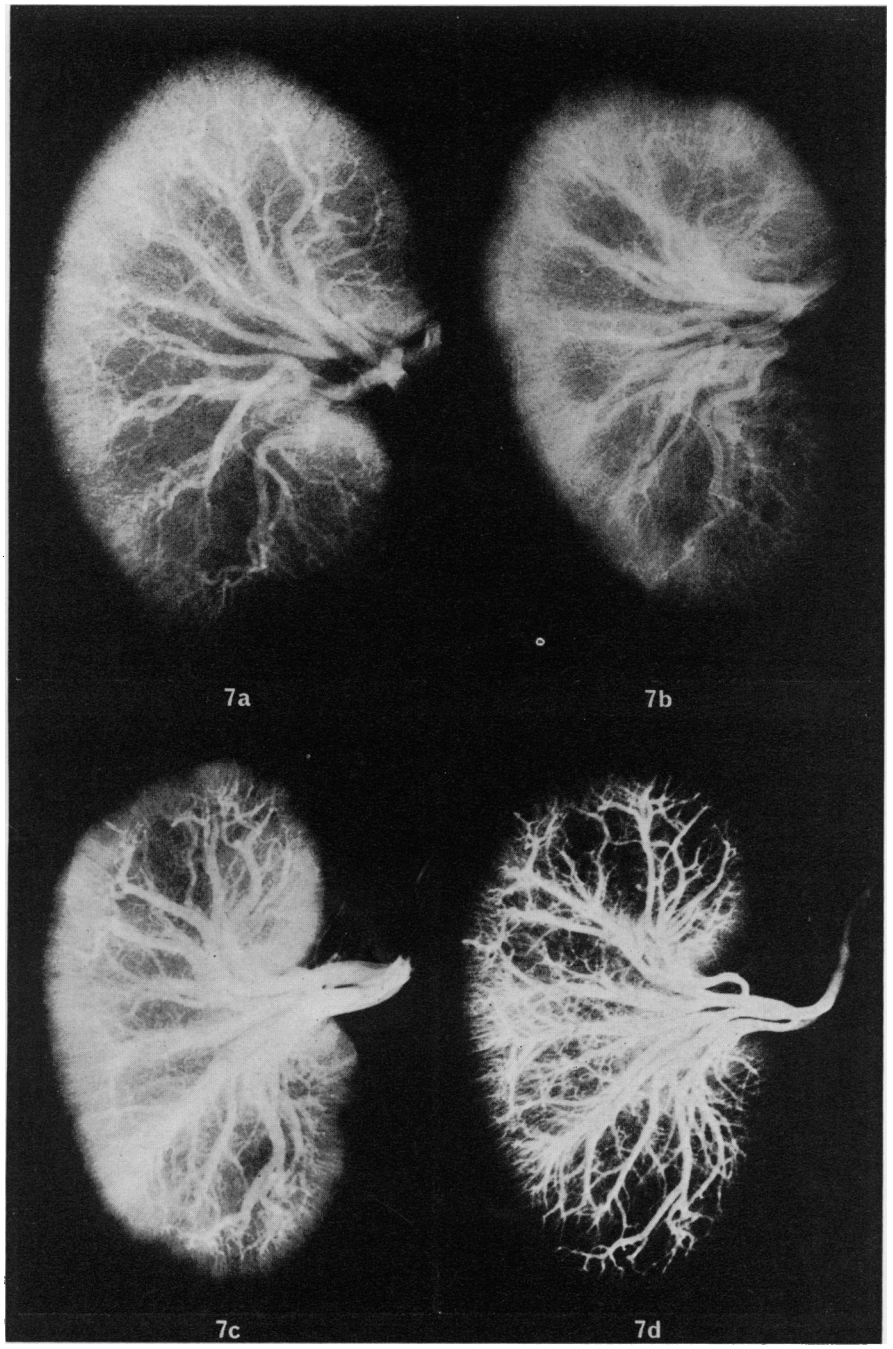
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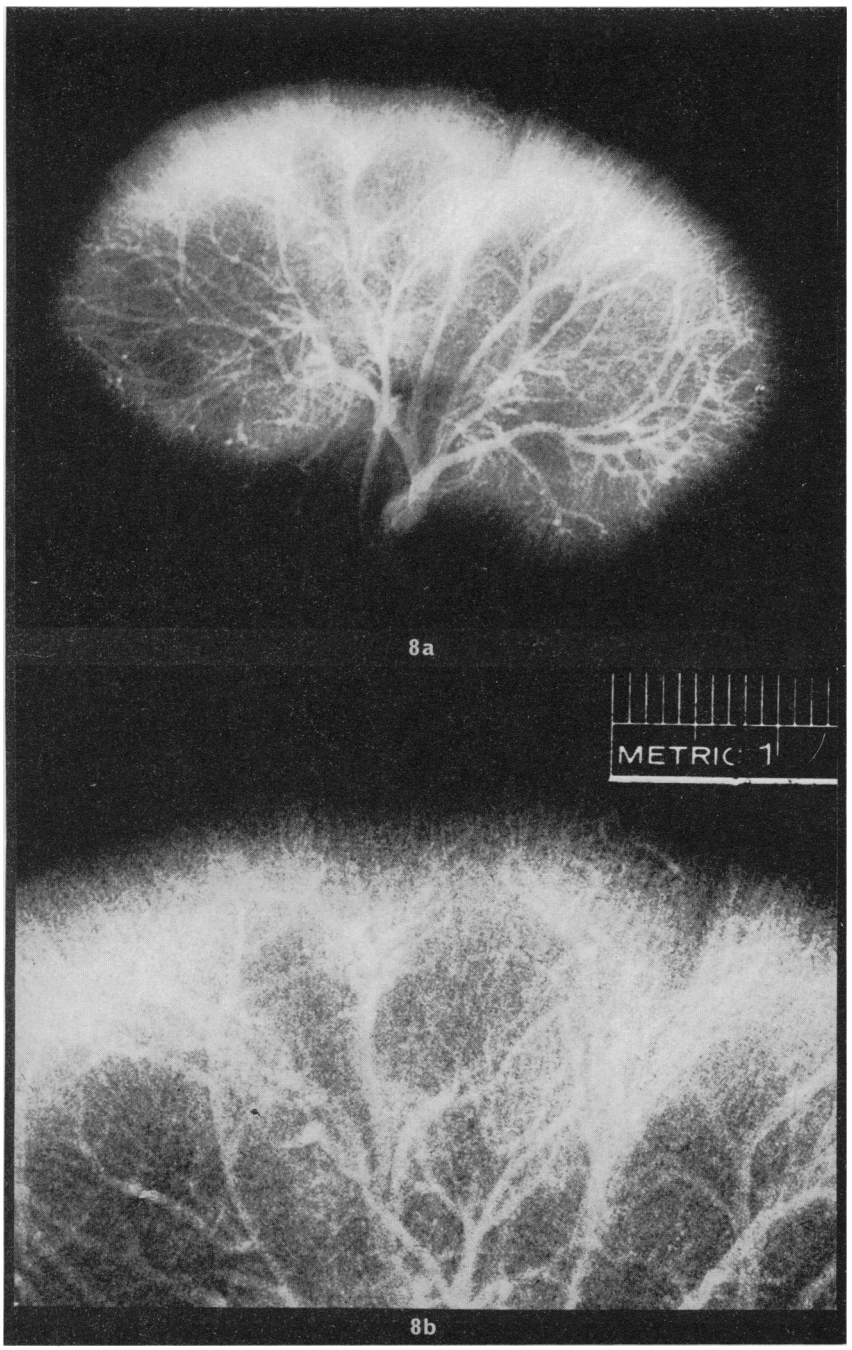
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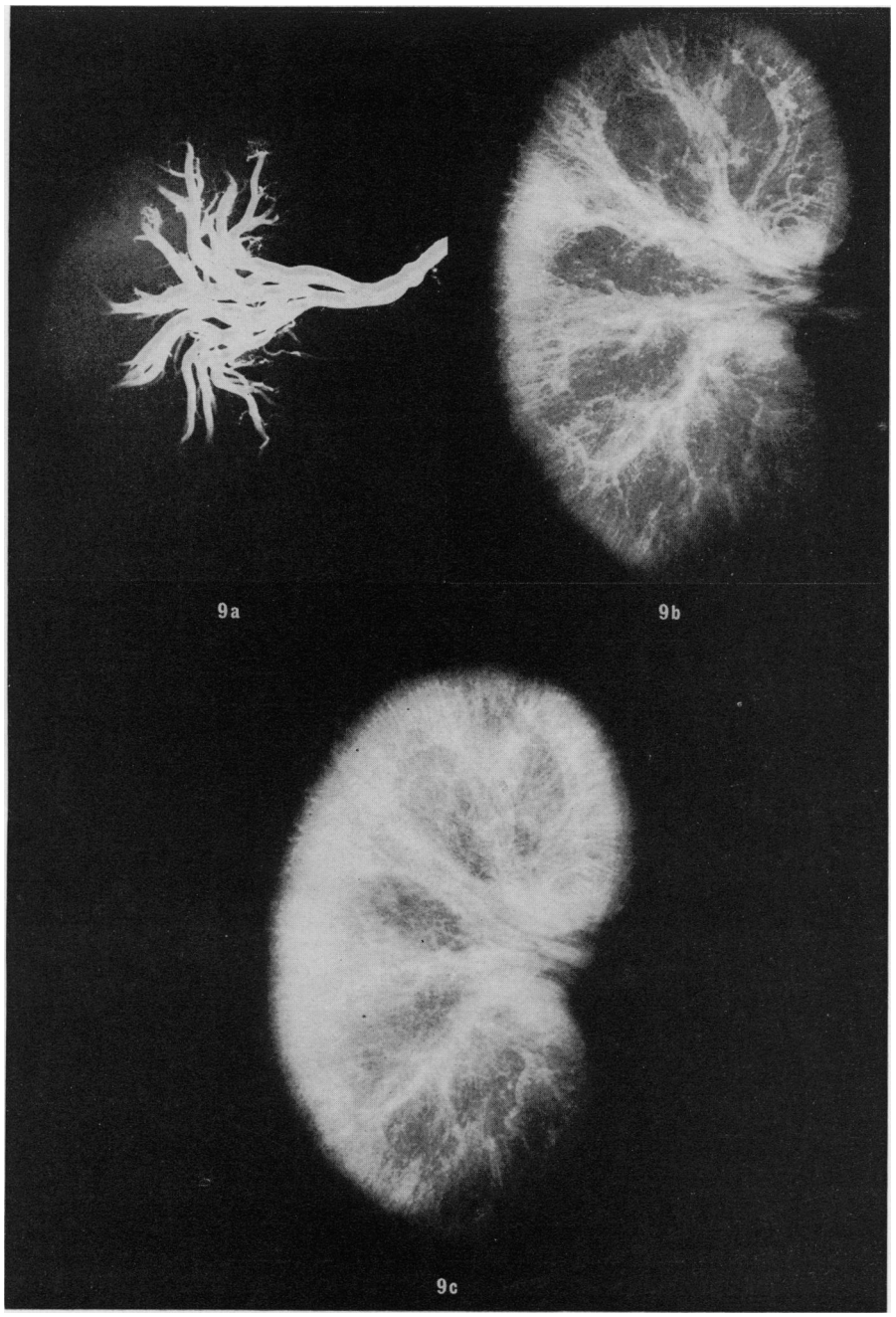
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effect which then partially reverses so that a water diuresis is only partially antagonised.

Hypertensin.—At a dose of 0·05 mg. Hypertensin produces violent afferent renal vasoconstriction with no flow beyond the interlobars in some instances (Fig. 9a). The effect on a water diuresis is an immediate anuria and then anti-diuresis lasting up to 50 min. (Fig. 10). The nature of this anti-diuresis is not clear since arteriograms show that renal perfusion recovers in 10–15 min. The anti-diuretic graph (Fig. 10) resembles that seen after an injection of Infundin (Dempster and Joekes, 1953). The possibility that Hypertensin stimulates ADH secretion is suggested. During the initial violent vasoconstriction it was observed that the capsule of the kidney wrinkled and the arterial blood pressure rose to over 200 mm. Hg. This feature is not observed when the renal artery is fully clamped. There are some transient systemic effects after a dose of Hypertensin of this magnitude. There is general unease and respiratory distress. Presumably this is due to vasoconstriction generally throughout the body. This stress may stimulate ADH secretion because the anti-diuretic effect lasts long after the outer cortical circulation has been restored.

If Frusemide (20 mg. i.v.) is injected prior to Hypertensin (0·05 mg. i.v.) it can be shown at 40 sec. (Fig. 9b), that the former drug can antagonize immediately the vasopressor effect of the latter. About 4 min. later (Fig. 9c) it can be demonstrated that the full vasodilator effect of Frusemide has been restored and the pressor effect of Hypertensin completely inactivated.

Mersalyl.—No evidence of vasoconstrictor activity was observed in renal arteriograms or renal excretion studies of PSP. The evidence appeared to indicate renal vasodilatation which is the exact opposite view of Hook *et al.* (1966). From the current data there is no evidence to support the view that Mersalyl is a renal vasoconstrictor.

Infundin (1 unit i.v.) is a remarkably strong renal vasopressor agent and delays the appearance of Phenol Red by 6–8 min. The anti-diuretic action of Infundin is due at first to renal afferent vasoconstriction and underperfusion of the outer cortex followed by the well-known increased tubular reabsorption.

Effect of Frusemide and Hypertensin on a water diuresis

Fig. 10 illustrates the mean of 3 experiments performed on each dog. The prolonged anti-diuretic action of Hypertensin may be due to ADH stimulation because adequate outer cortical perfusion is established within 15 min. of injecting Hypertensin. It appears that Frusemide is capable of antagonising Hypertensin and its diuretic property is due to vasodilatation and increased renal blood flow.

Effect of ergot preparations and Frusemide on a water diuresis

The ergot preparations effected an immediate diuresis which was immediately abolished by an injection of Frusemide.

Phenoxybenzamine and various forms of renal vasoconstriction

As assessed by arteriograph.—(a) There was no effect on a second-set reaction. (b) There was no antagonism to Hypertensin injected during a water diuresis. (c) There was no effect on vasoconstriction due to cold ischaemia. (d) There was

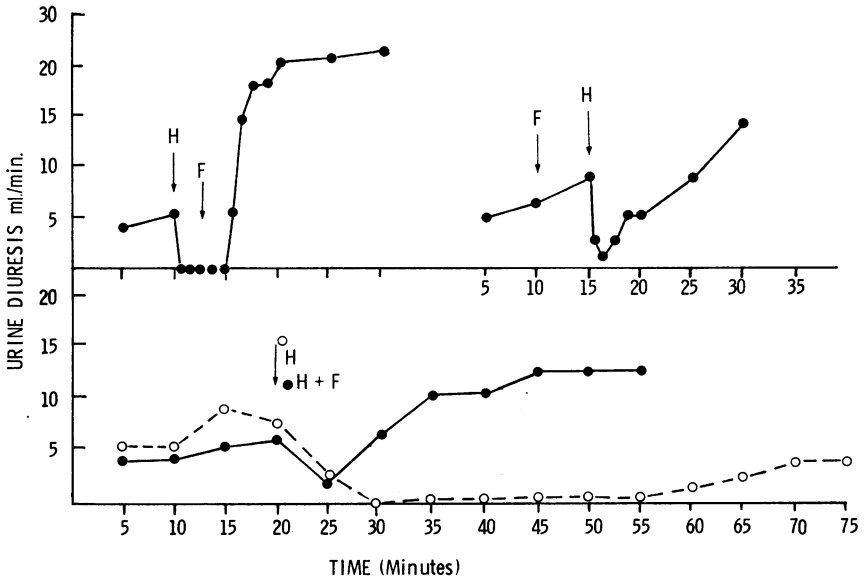

FIG. 10.—Water diuresis and anti-diuresis. Charts indicate the anti-diuretic action of Hypertensin (H) and the diuretic action of Frusemide (F). The prolonged anti-diuresis after Hypertensin suggests ADH has been stimulated because outer cortical perfusion is restored within 10–15 min.

no effect on vasoconstriction due to warm ischaemia. (e) There was no effect on vasoconstriction due to Infundin.

The effect of Propanolol and Hypertensin or Infundin on a water diuresis

The β-receptor blocker Propanolol (Inderal) was too slow acting to be effective against the immediate transient hypertension, renal vasoconstriction and anti-diuresis evoked by an intravenous injection of Hypertensin and other vaso-constrictors. It would appear, however, that Propanolol sometimes reduces the stress reaction of a Hypertensin injection. The anti-diuretic and vaso-pressor effects of Infundin were in no way antagonized by β-receptor blockade.

The effect of ganglion blockade (Ansolysen) and Hypertensin or Infundin on a water diuresis

Ansolysen (10 mg.) had no effect on the vasopressor effects of Hypertensin or Infundin.

Histology

Second-set kidneys showed widespread glomerular damage involving fibrinoid deposits and tubular necrosis (Dempster 1953*a*).

Kidneys after 2 hr warm ischaemia showed widespread tubular necrosis and stagnant red blood cells and platelets in the glomerular capillaries. Kidneys removed at various stages after cold preservation and transplantation showed varying degrees of tubular damage (Dempster *et al.*, 1964).

DISCUSSION

Failure to perfuse adequately the outer cortex is a phenomenon which occurs in response to various stimuli—pharmacological, immunological, nervous and ischaemic. In several conditions involving gross afferent vasoconstriction extending as far out as the interlobular vessels, vasodilators can reverse the vasoconstriction in the segments as far as the arcuates but fail to alter the state of the vessels perfusing the outer cortex. Fig. 4a, for example, illustrates this point. Procaine and papaverine have been used as vasodilators but the arteriogram shows that their action extends only to the arcuates leaving the outer cortical circulation underperfused. Nonetheless, Procaine and Frusemide are of real value in promoting eventual outer cortical perfusion in kidneys which have been preserved at 4° for several hr (Fig. 5). Even when the vasoconstriction of the afferent arterioles and interlobulars is not maximal, vasodilators are usually unable to reverse immediately the spasm due to cold. Outer cortical vasoconstriction due to other causes can be reversed by appropriate pharmacological means. As an example one can refer to the action of Frusemide in being able to cancel an established vasoconstriction and anti-diuresis due to an injection of Hypertensin (Fig. 9a) and in being able to promote extra flow in the outer cortex after cold preservation providing severe tubular damage has not occurred. It has been established by Hook *et al.* (1966) that Frusemide is a renal vasodilator and increases total renal blood flow. This function has been confirmed in the current experiments (Fig. 9b, c) and further, it has been shown that Frusemide is a renal vasodilator of greater potency than Hypertensin is a vasoconstrictor. This probably explains its beneficial action in renal hypertension. Fig. 9b, c, illustrate the degree of renal vasodilatation reached in spite of an injection of 0·05 mg. Hypertensin and one can observe the excellent outer cortical perfusion. This is at variance with the autoradiographs presented by Birtch, Sakheim, Jones and Barger (1967) claiming that Frusemide reduces juxta-medullary flow; the illustrating autoradiographs appear to involve artefacts because even the control at 60 sec. shows inadequate cortical flow. It would be a difficult task to explain the marked diuretic and natriuretic effect of Frusemide on any basis other than an increased outer cortical perfusion.

The statement made by Hook *et al.* (1966) that the mercurial diuretics are vasoconstrictors and increase renal vascular resistance has not been confirmed in the current experiments. By arteriography, PSP excretion time and percentage during water diuresis no evidence of vasoconstrictor ability has been observed.

So far as the second-set kidney reaction is concerned no agent has been found to overcome the intense outer cortical vasoconstriction (Fig. 2b, 3a) even when all the usual precautions have been taken to ensure good capillary flow after transplantation—*viz.* adequate prehydration, hydrocortisone-saline i.v. infusion during transplantation and mannitol or Rheomacrodex (Pharmacia) infusion immediately after transplantation or even when Arvin has been infused and an incoagulable state established prior to opening the new circulation (Pineo *et al.*, 1970). This certainly sets the second-set reaction apart from a reaction such as the Shwartzman reaction although similar vasomotor changes occur and similar parenchymal damage ensues. The gross non-specific nature of the second-set reaction has been discussed elsewhere (Dempster, 1969).

The factors limiting the various grades of outer cortical vasoconstriction have not been determined. So far as a first-set rejection of a kidney transplant is

concerned, the main factor in precipitating outer cortical vasoconstriction is a continuing increased vascular permeability maintaining a continuing increase of venular pressure (Dempster, 1970a). In other situations, as in the immediate phase after transplanting a cold preserved kidney or after warm ischaemia for 2 hr, the degree of outer cortical vasoconstriction is not maximal as can be demonstrated by injecting noradrenaline into such kidneys; Fig. 7c, d, illustrates that the degree of outer cortical vasoconstriction was not maximal in the second-set reaction but yet sufficient to induce acute renal failure in the kidney illustrated. The critical level of outer cortical perfusion below which renal failure occurs is, therefore, difficult to assess and, at present, impossible to measure. The current experiments have demonstrated that maximal vasoconstriction is not required to precipitate anuria and this raises the problem of which arterioles and glomeruli are excessively reactive to the various vasoconstrictor stimuli. This complicated subject will be considered in a subsequent communication.

These experiments have revealed many examples of kidneys showing apparently good filling of large areas of the outer cortex interspersed with small areas of underperfusion and yet associated with severe oliguria or anuria. In such kidneys, the total renal blood flow may be reduced by 20–40 per cent and in one exceptional case there was no reduction from the normal total flow although the kidney was anuric due to outer cortical vasoconstriction (Pineo *et al.*, 1970). It has not been possible in these experiments to measure the speed of flow and the percentage of total flow to the outer cortex of those kidneys rendered oliguric or anuric although it was possible to observe a delayed emptying time. These factors are not easily measured although attempts by techniques involving ^{133}Xe (Barger, 1966) have been made but these are open to serious mathematical criticism. However, the ^{133}Xe technique appears to indicate that reduction in speed and percentage flow are important factors. The apparently clear cut results presented by Rosen, Truniger, Kriek, Murray and Merrill (1967) are in conflict with the current simple experiments involving the intravenous injections of Priscol. This substance is a potent renal vasoconstrictor and exerts its effect, like adrenaline, on the whole extent of the arterial tree but without changing actual distribution except transiently when there is an initial outer cortical exclusion. The effect of this substance is to reduce markedly total renal blood flow and reduce the percentage flow to the outer cortex. This leads to an anti-diuretic effect only and not oliguria or anuria. Adrenaline or nor-adrenaline, on the other hand, has more dramatic although momentary anti-diuretic effects and yet arteriograms show that only focal areas of underperfusion occur momentarily (Dempster and Joekes, 1955). Although the complete details are not established a general rule emerges that any disturbance to a certain critical level of outer cortical perfusion will limit the excretion of salt and water. Any further disturbance will lead to severe oliguria or anuria. This is precisely the disturbance which overtakes a second-set kidney sooner or later and certainly within a few hr. of being transplanted to a sensitized recipient.

Changes from the normal distribution of renal blood flow are frequently referred to as redistribution (Aukland, 1968). This is a confusing term because it implies the re-routing of blood through areas previously not perfused. This is manifestly not true but the origin of this confusion is probably in the original report of the Oxford shunt (Trueta, Barclay, Daniel, Franklin and Prichard, 1947) which considered that when shunting took place the normal volume of renal blood was

re-routed *via* the vasae rectae into the medulla which are too narrow in any case. When vasoconstriction and underperfusion of the outer cortex occur there is, in fact, a fall in total renal blood flow. There is no redistribution but a reduction in distribution whereby the outer cortex is denied its proper percentage of blood volume at a given speed while the juxta-medullary zone continues to receive an increased, normal or subnormal blood flow.

There is still some debate about the actual site of action of adrenaline and nor-adrenaline in the kidney. Smith (1951) reviewed evidence for the venules. Aukland (1968) showed that, during infusions of adrenaline, glomerular filtration rate was maintained in spite of considerable reduction in total renal blood flow and that this suggested post-glomerular vasoconstriction. There are no α-adrenergic fibres on the efferent arterioles (Norvell, Weitsen and Shepek, 1970) and hence, one can rule out this segment as the site of action. It was clearly demonstrated by Dempster and Joekes (1955) that intravenous injections of adrenaline or nor-adrenaline up to 80 μm. effected general afferent vasoconstriction with areas of markedly reduced outer cortical perfusion. Since there are α-adrenergic receptors on the capacitance vessels, it is possible that adrenaline acts first by increasing the venular resistance which precipitates afferent vasoconstriction (Dempster, 1970a) together with the effect on α-adrenergic receptor sites of the afferent system and which now comes to dominate the haemodynamic state so long as the effect of the drug lasts. However, as the result of reduced afferent flow the venular resistance may rise, by some unknown signal to prevent too low a pressure in the renal vein developing (Pate and Estes, 1968), and so the vascular resistance rises. This is, perhaps, the post-glomerular resistance which Aukland (1968) detected. With a single injection of adrenaline or nor-adrenaline the vasoconstrictor effect is momentary (Dempster and Joekes, 1955) and the anti-diuretic effect, by exclusion of outer cortical glomeruli from perfusion, is also momentary.

It has been shown that, on releasing renal blood flow after 2 hr of warm total ischaemia, increased renal resistance occurs and Hinshaw, Archer, Parry and Shires (1970) have concluded that this is due to vasoconstriction. This has been confirmed arteriographically in the current experiments but the vasoconstriction has been shown to be confined to the outer cortex (Fig. 6a). This can account for the modest reduction in total renal blood flow in such anuric kidneys. Hinshaw *et al.* (1970) have postulated that the renal vasoconstriction evoked after a period of warm ischaemia is due to the release of a pressor agent. In the current experiments, the pressor agent released, if any, was not renin because neither phenoxybenzamine nor Frusemide exerted any reversal effect and arteriography showed no change in the appearance of the established outer cortical vasoconstriction. Renal failure in these experiments is due, in the immediate phase after releasing the circulation, to failure of outer cortical filtration and later on due to this factor and the tubular necrosis which has developed as a result of inadequate outer cortical flow.

There is a close similarity between the afferent vasoconstriction observed in the early phase of acute rejection of first-set kidney allotransplants and the early phase of second-set reaction. Within reasonable limits the vasoconstriction of the first-set reaction can be reversed by steroids which aid in restoring proper vascular permeability (Dempster, 1970*a*). Steroids have no effect, however, in reversing the vasoconstriction of the early phase of the second-set reaction. For this and

several other reasons the second-set reaction is not merely an exaggerated response by the same factors which mediate the first-set reaction (Dempster, 1955). This difference illustrates again that there are many and varied causes of outer cortical vasoconstriction and reversal of this state is contingent upon applying the proper pharmacological antagonist.

There is a certain similarity between the outer cortical vasoconstriction induced by 2 hr of warm ischaemia and the early phase vasoconstriction of the second-set kidney transplant reaction. Neither respond to pharmacological vasodilators but the histological consequences differ in one important respect. Fibrin deposits in the glomeruli are characteristic of the second-set reaction whereas fibrin is seldom seen early after damage caused by warm ischaemia. Fibrin deposits in the glomeruli are not the cause of second-set reaction but the result of some severe immunological reaction which initially manifests itself in vasoconstriction. If the second-set reaction does not supervene in a kidney transplanted to a sensitized animal the vasoconstriction does not occur and fibrin deposition does not occur either (Pineo *et al.*, 1970). It is possible that high energy antibodies could cause severe vascular spasm of the afferent arterioles and glomerular capillaries but the nature of the next step towards the deposition of fibrin is not clear, although it could be postulated that a local breakdown of fibrinolytic activity occurs in the glomerular cells as a result of the second-set reaction but not as a result of warm ischaemia and other non-specific causes of an upset of renal haemodynamics (Dempster, 1969). In the early phase of Masugi nephritis there is fibrin deposition in the glomeruli and some evidence that it is usually only temporary since, it is thought, the glomerular cells quickly regain their fibrinolytic activity (Shigematsu, 1970). If this function is not regained quickly massive thrombi develop in the glomeruli and death occurs within 30 hr. The main difficulty in accepting this postulate lies in the fact that fibrin deposits occur in second-set kidneys even when the recipient has been reduced to an incoagulable state prior to the transplantation (Pineo *et al.*, 1970).

Fibrin deposits in the glomeruli of a second-set kidney lead to secondary renal damage but in other disease conditions involving a similar pathology absolute anuria is not encountered and in some instances the fibrinoid changes can resolve. The second-set reaction, on the other hand, moves inexorably to a stage at which afferent vasoconstriction is so severe that no blood enters the kidney and total irreversible damage results (Pineo *et al.*, 1970).

Factors released by the second-set reaction initiate the reactivity of the outer cortical vessels in a manner similar also to the effect produced by moderate doses of drugs like adrenaline, Priscol and Hypertensin. But the pharmacological blockers of these drugs are valueless in abrogating the second-set kidney reaction which suggests that the endothelium has been paralysed and organic damage of the vessels walls has occurred or that α and β-adrenergic stimulation are not involved. Indeed, no combination of these drugs, immunosuppression and an incoagulable state has provided any encouragement that second-set reactions can be consistently abrogated. Caution should be exercised in claims for abrogation since, as previously shown (Pineo *et al.*, 1970), a second-set kidney reaction is not an absolute consequence of sensitization or even hypersensitization and the factors involved in this mystery are worth further study.

What has emerged in these current experiments is the marked reactive nature of the vessels of the outer cortex of kidneys involved in a wide variety of experi-

mental conditions. While the renal afferent vascular tree usually responds *in toto* to a stimulus like adrenaline or Hypertensin, there is an added intensity in the outer cortical vessels. In seeking a rational explanation for the independent behaviour of the outer segment of the renal arterial tree one may regard it as a mechanism evolved to enable the kidney to handle salt and water according to given stimuli. Is the normal renal peripheral vascular resistance, in effect, largely a renin effect which would explain its main concentration around the glomeruli of the outer cortex? This would seem a reasonable deduction from the observation that salt loading decreases renal vascular resistance (Earley and Friedler, 1965) and this is probably effected by reduced renin secretion. Thus, a delicate but highly reactive mechanism evolved to meet the needs of salt conservation can be easily disturbed by a number of stimuli quite unrelated to renin or to salt conservation with sometimes grave consequences to renal function.

General effect of vasoconstrictor drugs

The general effect of all vasoconstrictors is to delay by 4–8 min. the excretion rate of phenol red. This correlates with the arteriographic evidence that outer cortical underperfusion has occurred. There is some evidence (Brooke and Robinson, 1970) that ergot preparations are venoconstrictors as well as afferent vasoconstrictors. This is similar to the effects of an injection of adrenaline. It is suggestive that all vasoconstrictors are partly venoconstrictors and that venoconstriction, by increasing the peripheral vascular resistance, triggers off afferent vasoconstriction in addition to the direct vasoconstrictor effect of the drugs concerned.

The rate of excretion of phenol red (normally appears 2–2½ min. after injection) is delayed by all vasoconstrictors. Infundin can delay the excretion up to 8 min. Since arteriography can demonstrate that the cause of this delay in phenol red excretion is transient outer cortical underperfusion due to afferent vasoconstriction it is tempting to conclude that the measurement of effective renal plasma flow is, in reality, a measurement of outer cortical nutrient flow only. It has been demonstrated that phenol red excretion is diminished or abolished when outer cortical underperfusion occurs in first-set kidney rejection (Dempster, 1970*a*) and in second-set kidney rejection (Pineo *et al.*, 1970). This also suggests that only the nephrons derived from outer cortical glomeruli are concerned with excretion of the glomerular filtrate.

An i.v. injection of Frusemide can abolish the vasoconstrictor effect of all pharmacological vasopressors so far tested. Frusemide is valueless in abrogating the vasoconstriction induced by warm total renal ischaemia of 2 hr duration, cold ischaemia of 20 hr duration if severe and first-set acute rejection. The α-adrenergic blockers and Procaine (smooth muscle paralyser) are also ineffective in such situations. Steroids are effective in relaxing the vasoconstriction of first-set acute rejection because vascular permeability becomes stabilized but these drugs are ineffective against the vasoconstriction induced by warm and cold ischaemia and the second-set reaction. Although the vasoconstriction induced in the latter 3 models is seldom maximal at the onset it is sufficiently strong to evoke oliguria or anuria. The vasoconstriction induced by the second-set reaction, on the other hand, is not usually maximal at its onset but over the subsequent hours becomes always progressively more intense until only medullary blood, and sometimes not even that, is permitted through the rejected kidney.

The vasoconstriction of the second-set reaction is akin to that induced in renal cortical necrosis in the human and its mediating factors are equally obscure. The vasoconstriction evoked by the Shwartzman reaction can be abrogated by denervation and α-adrenergic blockers and requires the presence of the adrenals and these aspects differentiate it from the second-set reaction which has not been abrogated, so far, by any agent which vasodilates, paralyses smooth muscle or reduces peripheral vascular resistance. The violence of the second-set reaction can be mimicked by a technical haemodynamic upset of the renal circulation (Dempster, 1954*a*, 1969) in an autotransplanted kidney and the differential diagnosis can be quite difficult if a second-set kidney does not reveal fibrin deposition.

The nature of second-set vasoconstriction

Second-set vasoconstriction has some aspects in common with vasoconstriction evoked by warm and cold ischaemia *viz.* unaffected by α-receptor blockers and smooth muscle paralysers. How is such a severe and irreversible vasoconstriction mediated? If it were due to mural oedema it would take some hr to develop and one would expect some amelioration with pre-transplant high steroid dosage but steroid offers no protection (Dempster, 1953*b*). If it were due to release of vasoactive kinins one would expect that anti-histamine drugs would reduce the severity but they do not. If lymph node permeability factor were involved one would expect that Indomethacine, given i.v., would modify the reaction but it does not. In fact, no drug has so far modified second-set kidney reactions the first signs of which are vasomotor changes in the outer cortex.

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