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# AUTOREGULATION OF PLASMA FLOW IN THE ISOLATED PERFUSED RAT KIDNEY

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SUMMARY

1. Autoregulation of renal plasma flow, by which flow remains constant despite changes in perfusion pressure, was studied in the isolated, perfused kidney of the rat.

2. Autoregulation did not occur in preparations perfused with a protein-free medium consisting of a balanced ionic solution resembling rat plasma in which 3% polyvinylpyrrolidone replaced the plasma proteins, changes in perfusion pressure over the normal autoregulatory range 100–150 mmHg produced a corresponding and linear change in venous outflow and no consistent change in renal vascular resistance.

3. Addition of human serum (5%, v/v) to the medium restored autoregulation; changes in perfusion pressure in the range 100–150 mmHg resulted in a stable plasma flow and a linear change in renal vascular resistance. The addition of bovine serum albumin (3 g/l.) to the protein-free medium restored autoregulation to a similar degree.

4. In kidneys perfused with the protein-free medium, the sensitivity of the renal vasculature to the vasoconstrictor drugs epinephrine and angiotensin II was only 1/40 the level seen in those kidneys perfused with media containing serum or albumin.

5. The experiments show that in the isolated, perfused kidney, autoregulation of plasma flow is not dependent on the presence of the globulin, angiotensinogen, in the perfusion medium; and suggest that failure of autoregulation in kidneys perfused with a protein-free medium could be attributed to the rapid decline in the sensitivity of the vascular smooth muscle to constrictor stimuli.

## INTRODUCTION

Variation in arterial pressure over a wide range of values produces very little change in renal blood flow. Thus changes in renal arterial pressure are accompanied by directly proportional changes in renal vascular resistance. The phenomenon is described as autoregulation of renal blood flow. In the dog, variations in arterial pressure over the range 80–180 mmHg cause little change in the rate of renal blood flow (Selkurt, 1951; Thurau, 1964). In the rat, the autoregulatory range is 100–150 mmHg (Arendshorst, Finn & Gottschalk, 1975).

Three mechanisms have been proposed to account for autoregulation: variations of blood viscosity, produced by a variable degree of plasma skimming (Pappenheimer & Kinter, 1956); variations in interstitial pressure, resulting passively from changes in hydrostatic pressure (Hinshaw, 1964); and variations in the tone of the vascular

smooth muscle (Thurau & Kramer, 1959). Plasma skimming was eliminated as an explanation when it was shown that autoregulation could occur in kidneys perfused with cell-free media (Waugh, 1964; Weiss, Passow & Rothstein, 1959). A direct effect of intrarenal interstitial pressure has seemed improbable since it was shown that autoregulation was abolished after smooth muscle contraction was prevented by the action of papaverine despite concomitant changes in intrarenal interstitial pressure (Thurau & Kramer, 1959). These experiments implicated active contraction of smooth muscle as the means by which autoregulation is achieved.

Controversy still exists as to how changes in renal arterial pressure affect the vascular smooth muscle. One explanation is that changes in smooth muscle tone represent a direct response to changes in vessel wall tension – the myogenic theory (Thurau & Kramer, 1959; Folkow, 1964; Thurau, 1964). The other theory is that changes in smooth muscle tone result from the variable formation of a vasoconstrictor substance, the juxta-glomerular theory (Thurau, 1967). According to the myogenic theory, changes in pressure in the vessel cause changes in wall tension, smooth muscle cells are stimulated to contract more frequently (Bülbring, 1955) and resistance to flow is increased (Folkow, 1964; Johansson & Bohr, 1966). According to the juxtaglomerular hypothesis, variations in arterial pressure cause changes in the release of renin from the cells of the juxtaglomerular apparatus and hence changes in the level of angiotensin II.

Since the juxtaglomerular mechanism requires the presence of circulating angiotensinogen, and the myogenic mechanism does not, the isolated, perfused kidney could be used to test these hypotheses, in that extrarenal factors, including the composition of the perfusion medium, are controlled and extrarenal sources of angiotensinogen can be eliminated. The rapid flow rates which occur in the perfused preparation would wash out any renal angiotensinogen which is not intracellular or firmly bound to the cell membrane.

This paper presents the results of experiments which tested the autoregulatory capacity and vascular reactivity of rat kidneys perfused in the presence and absence of extrarenal angiotensinogen.

## METHODS

A detailed description of the apparatus and methods is given elsewhere (Bullivant, 1978).

Sprague-Dawley rats of both sexes and weighing 200–400 g were anaesthetized with I.P. pentobarbitone (60 mg/kg body wt.). The kidney was perfused via a catheter placed in the abdominal aorta below the renal artery. All side branches of the aorta except the appropriate renal artery were tied off. Perfusion started before the aorta above the renal artery was tied, so that at no time was the flow to the kidney interrupted or restricted. The venous outflow from the kidney was passed to a fraction collector via a catheter in the vena cava. The ureter was catheterised with a polythene tube (length 3 cm, o.d. 0·5 mm) or merely cut.

The basic perfusion medium had the following composition: NaCl 115 mM, $NaHCO_3$ 25 mM, KCl 5 mM, $KH_2PO_4$ 0·4 mM, $CaCl_2$ 1 mM, $MgSO_4$ 1 mM, Na lactate 3 mM, urea 5 mM, glucose 15 mM, polyvinylpyrrolidone (mol. wt. 44,000) 30 g/l., inulin 250 mg/l. It will be called the 'protein-free medium'. This protein-free medium was varied by the addition of either 50 ml./l. of human serum, called the 'serum-containing medium', or 3 g/l. bovine serum albumin (Sigma Fraction V) (the same weight of albumin as contained in the 50 ml. human serum), called the 'albumin-containing medium'. The medium was filtered before use through a filter of pore size 0·8 $\mu$m, was held in a reservoir at 42 °C (to allow for cooling between the bath and

the kidney) and gassed with a mixture of 95% $O_2$, 5% $CO_2$ for 1 hr before the experiment. The medium was passed through another filter of 0·8 $\mu$m pore size en route to the kidney.

Perfusion pressure was measured by a pressure transducer (Statham, model P23Db) connected just proximal to the arterial catheter. The transducer was calibrated and checked using a standing saline column. The pulsatile perfusion pump (Harvard Model 1405) was equipped with a speed modulator which could be set by means of a second pressure transducer to maintain any chosen perfusion pressure, irrespective of changes in renal vascular resistance.

Renal plasma flow was measured by continuous collection of the venous effluent into pre-weighed tubes in the fraction collector, set for half minute collections.

Renal vascular resistance was calculated as perfusion pressure/venous outflow, i.e. mmHg. $ml.^{-1}.min^{-1}.g$ kidney $wt.^{-1}$.

*Experimental procedure*

Perfusion was set up at a mean pressure of 125 mmHg with a pulse pressure of 15 mmHg and maintained at this pressure level for 20 min, by which time the venous outflow rate was constant and the effluent free of blood. The mean perfusion pressure was then reduced in 20 mmHg increments to 40 mmHg and the flow rate determined for 3–5 min at each new level of pressure. The pressure was then restored to 125 mmHg and a new stable flow rate determined before the pressure was raised incrementally to about 190 mmHg. The flow rate at a perfusion pressure of 125 mmHg just before each series of pressure changes was taken as the control value and used to calculate the percentage changes in flow and in renal resistance observed to occur with each subsequent change in perfusion pressure.

*Determination of the sensitivity of the renal vasculature to vasoconstrictor drugs*

The sensitivity of the perfused kidney preparation to the vasoconstrictor drugs angiotensin II (Hypertensin CIBA) and epinephrine (adrenaline bitartrate) was tested by monitoring the response to graded doses of the drugs in kidneys perfused with the three different media. The reactivity of the smooth muscle was tested by injection of graded doses of barium chloride. Each kidney was perfused for 20 min at a perfusion pressure of 125 mmHg, which was maintained constant throughout the experiment. The stable flow rate was determined and then a known amount of the test substance (in a volume of saline no greater than 1 ml.) was rapidly injected into the flowing medium just proximal to the kidney. The lowest flow rate for any half minute interval after injection was compared to the flow rate just before the injection and the result expressed as a percentage reduction in flow. A dose–response curve was plotted for each drug in kidneys perfused with the three media.

## RESULTS

### (1) *Autoregulation of plasma flow in kidneys perfused with the protein-free medium*

The results from experiments on five kidneys perfused with the protein-free medium are presented in Fig. 1 where the percentage change in venous outflow is plotted against the perfusion pressure; and in Fig. 2 where the percentage change in renal resistance is plotted against the perfusion pressure. Fig. 1 shows no evidence of autoregulation of renal plasma flow. The points lie close to a straight line. Thus there is no inflexion over the expected autoregulatory range 100–150 mmHg. This is corroborated by the data shown in Fig. 2; the resistance of the renal blood vessels remained relatively constant despite the changes in perfusion pressure.

### (2) *Autoregulation of plasma flow in kidneys perfused with the serum-containing medium*

The results from four kidneys perfused with the serum-containing medium are presented in Fig. 3, where the percentage change in venous outflow is plotted against perfusion pressure; and in Fig. 4 where the percentage change in renal vascular resistance is plotted against the perfusion pressure. Fig. 3 shows an inflexion in the

line indicating that the plasma flow is relatively constant over the pressure range 100–150 mmHg. Autoregulation is confirmed by Fig. 4, which shows a linear change in renal vascular resistance with change in perfusion pressure over the autoregulatory range. A 50% change in perfusion pressure caused a 26% change in renal vascular resistance.

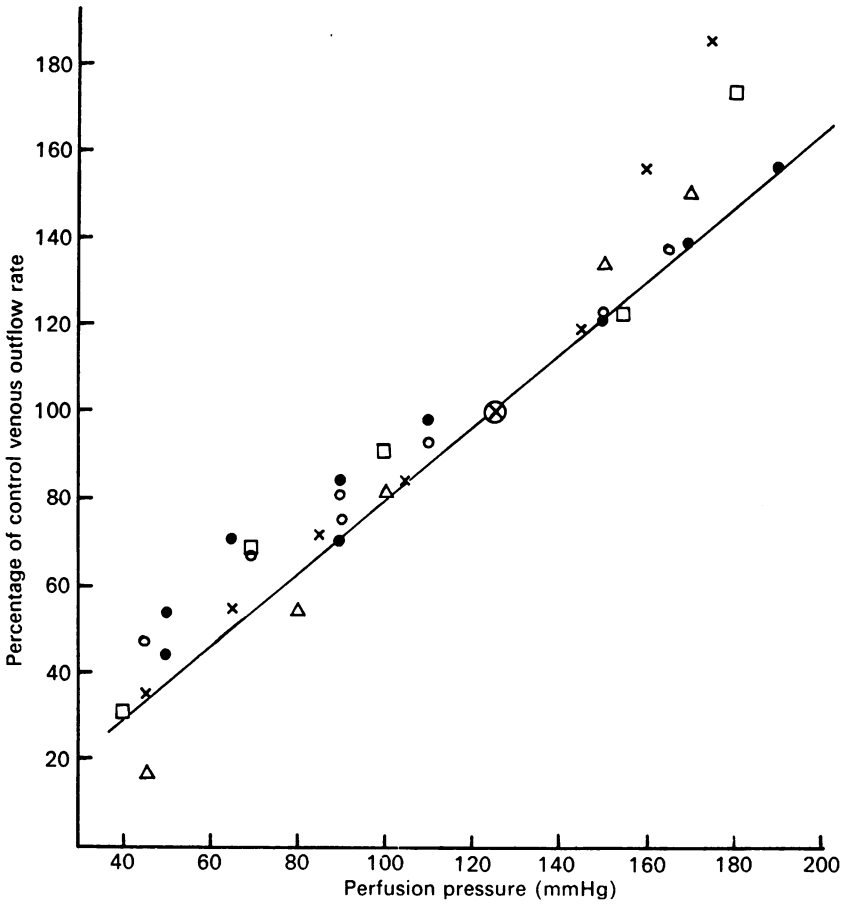


Fig. 1. The variation of venous outflow rate with perfusion pressure in kidneys perfused with the protein-free medium (the different symbols represent data from five separate experiments. ⊗, control venous outflow rate. The line is the best fit drawn by eye).

(3) *Autoregulation of plasma flow in kidneys perfused with albumin-containing medium*

The results from experiments on six kidneys perfused with the albumin-containing medium are presented in Figs. 5 and 6. Over the pressure range 100–150 mmHg, there is an inflexion in the slope of the line relating renal plasma flow to perfusion pressure. Renal vascular resistance changed linearly over the autoregulatory range, so that a 50% change in perfusion pressure produced a 25% change in renal vascular resistance.

(4) *Sensitivity of the renal vasculature to epinephrine, angiotensin II and barium chloride*

The dose–response curves to epinephrine of kidneys perfused with the protein-free medium, the serum-containing medium and the albumin-containing medium are shown in Fig. 7. The sensitivity to epinephrine was greatly reduced in kidneys perfused with the protein-free medium. 50% reduction in venous outflow required a single dose of 7·5 $\mu$g epinephrine in kidneys perfused with the protein-free medium,

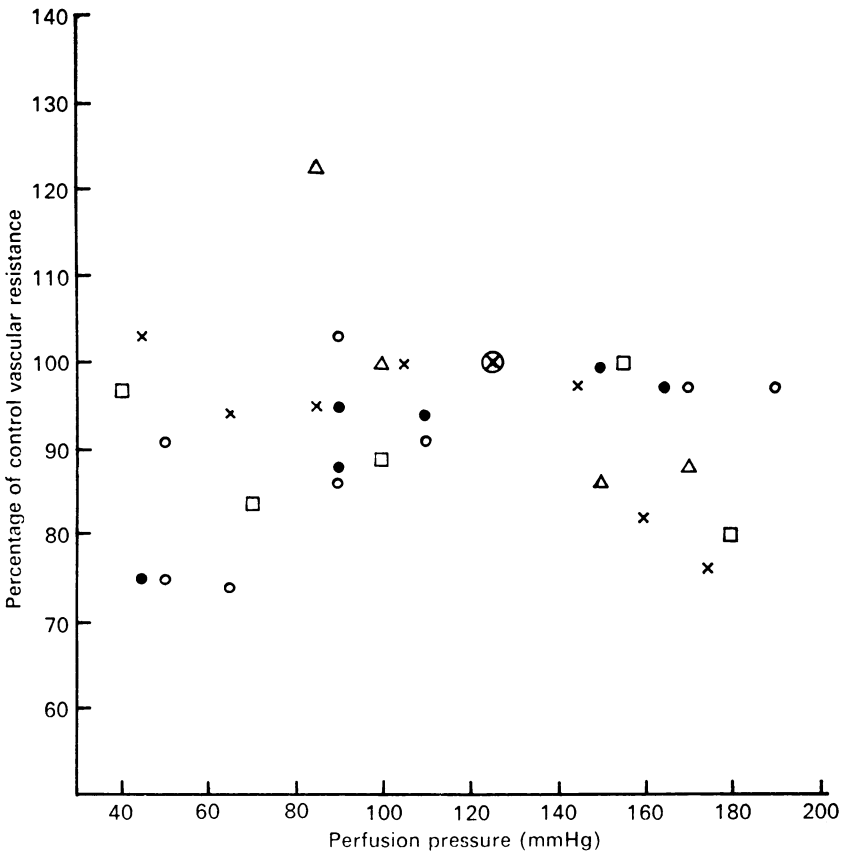


Fig. 2. The variation of renal vascular resistance with perfusion pressure in kidneys perfused with the protein-free medium (the different symbols represent data from five separate experiments. ⊗, control vascular resistance).

of 1·0 $\mu$g epinephrine in kidneys perfused with the albumin-containing medium and of 0·16 $\mu$g epinephrine in kidneys perfused with the serum-containing medium. The slope of the dose–response curve was similar in preparations perfused with media which contained serum or albumin, but in the case of albumin, the dose–response curve was shifted to the right. With the protein-free medium, the slope of the dose–response curve was reduced, but the threshold dose was unchanged.

The dose–response curves to angiotensin II are shown in Fig. 8. In kidneys per-

fused with the protein-free medium, the response to angiotensin II appeared to be small and independent of the dose. The addition of either serum or albumin to the medium increased the response which now varied with the dose injected. The response to angiotensin II was similar in both these series of preparations.

The vasoconstrictor response of kidneys perfused with the basic medium to repeated single doses of either drug varied with time, being maximal at the start of perfusion and declining to a constant value after 20 min perfusion. In kidneys perfused with the serum or protein-containing media, the vasoconstrictor response to a single dose of either drug remained constant over the whole time course of the perfusion.

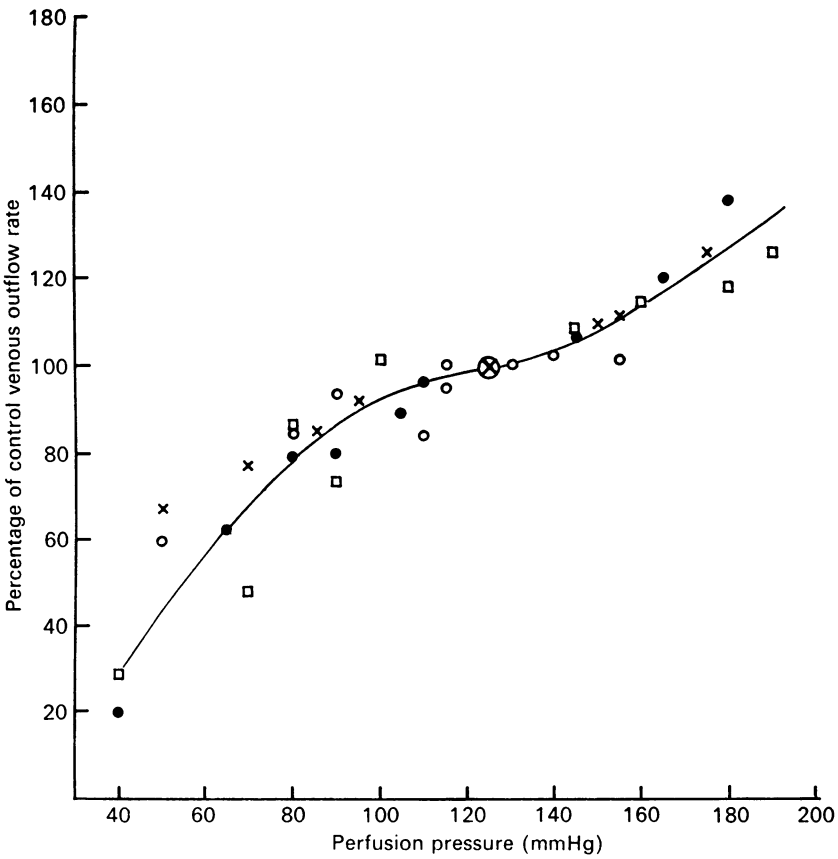


Fig. 3. The variation of venous outflow rate with perfusion pressure in kidneys perfused with the serum-containing medium (the different symbols represent data from four separate experiments. ⊗, control venous outflow rate. The line is the best fit drawn by eye).

Kidneys perfused with all three media responded to an injection of 25 $\mu$mole-barium chloride with a maximal vasoconstriction, causing a reduction of venous outflow by more than 80%. It is therefore evident that in preparations perfused with the basic medium alone, the smooth muscle of the renal blood vessels is capable of maximal contraction.

## DISCUSSION

The experiments show that after 20 min perfusion with the protein-free medium, the perfused kidney was unable to autoregulate plasma flow; a change in perfusion pressure did not produce a corresponding change in the resistance of the renal blood vessels.

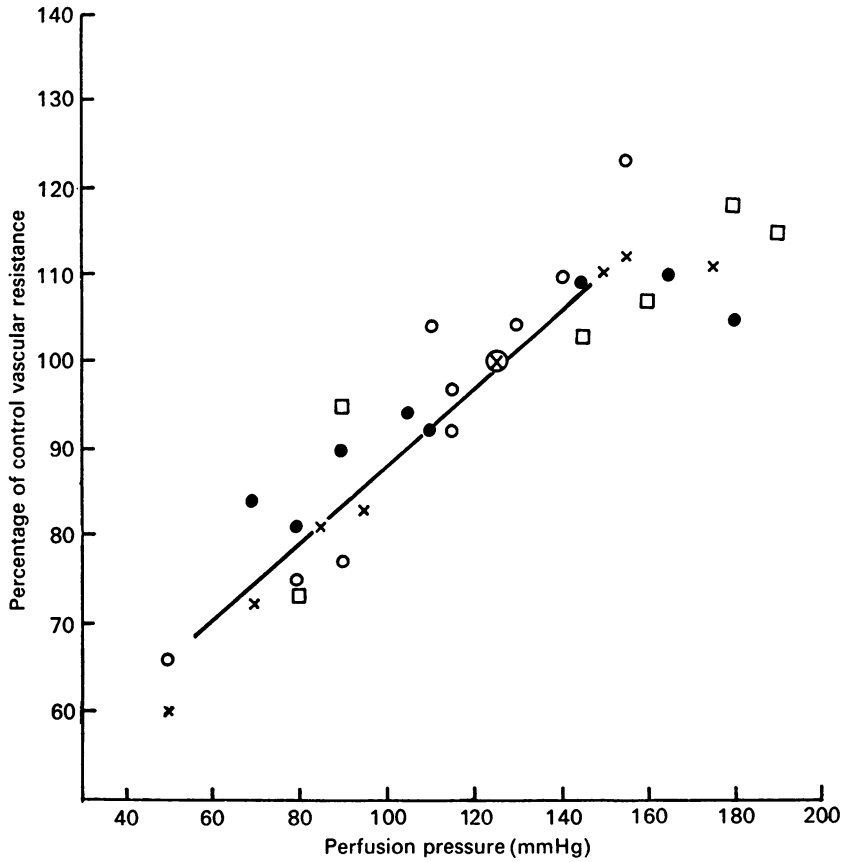


Fig. 4. The variation of renal vascular resistance with perfusion pressure in kidneys perfused with the serum-containing medium (the different symbols represent data from four separate experiments. ⊗, control vascular resistance. The line is the best fit drawn by eye).

If autoregulation of plasma flow is considered to involve the active contraction and relaxation of vascular smooth muscle, then autoregulatory failure could occur at any point in the chain of events whereby an applied stimulus is received, transmitted from receptor to effector and elicits a response from the effector. Different mechanisms are proposed in the myogenic and juxtaglomerular hypotheses. In the myogenic hypothesis, the pressure sensor would be the muscle cell membrane, the transmitter stage the coupling of excitation to contraction, and the effector the contractile elements of the smooth muscle. In the juxtaglomerular hypothesis, the receptor is situated in the juxtaglomerular apparatus; the transmission of the stimulus

is achieved by the variation in the release of renin and hence in the ultimate production of angiotensin II and its action on the smooth muscle; and the effector mechanism is the contractile element of the smooth muscle. The most obvious difference between the two theories, and the easiest to test, is the need for the presence of a substrate for renin.

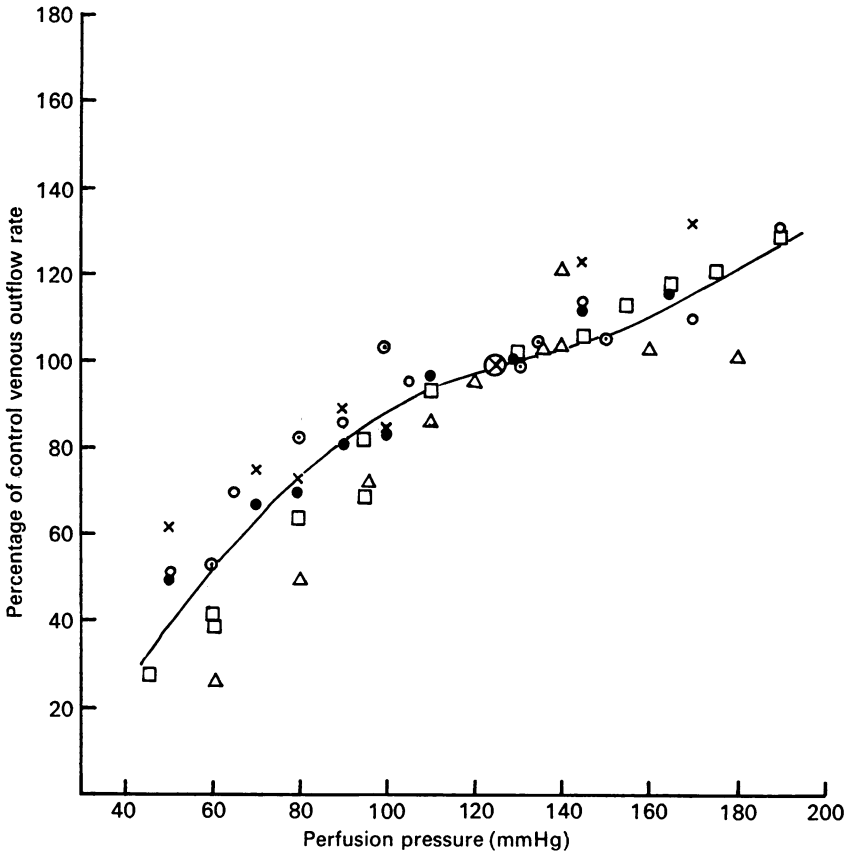


Fig. 5. The variation of venous outflow rate with perfusion pressure in kidneys perfused with the albumin-containing medium (the different symbols represent data from six separate experiments. ⊗, control venous outflow rate. The line is the best fit drawn by eye).

Kidneys perfused with a medium containing 5% human serum (v/v) showed a linear change in renal resistance with perfusion pressure. This agrees with the results of Waugh (1964) who found that the capacity for autoregulation declined rapidly in kidneys perfused with a plasma-free medium, but that it could be restored by adding 20% (v/v) plasma to the perfusion medium. The changes in renal resistance are comparable with those seen by Arendshorst *et al.* (1975) in the rat kidney *in vivo* when the concentration of angiotensinogen is normal and not reduced to the low levels used in these experiments.

However, as well as providing a renin substrate, the addition of human serum could also have had an effect on other events in the autoregulatory chain, that is on

the sensitivity of the receptor mechanism, the coupling of excitation to contraction at the muscle level or the ability of the smooth muscle to contract. Further experiments were carried out to test the sensitivity of kidneys perfused with the protein-free and serum-containing media to two vasoconstrictor drugs with differing action on smooth muscle cells, angiotensin II and epinephrine, and to the barium ion. Angiotensin II causes smooth muscle to contract by its action on the cell plasma membrane which eventually causes the release of calcium ions from the sarcoplasmic

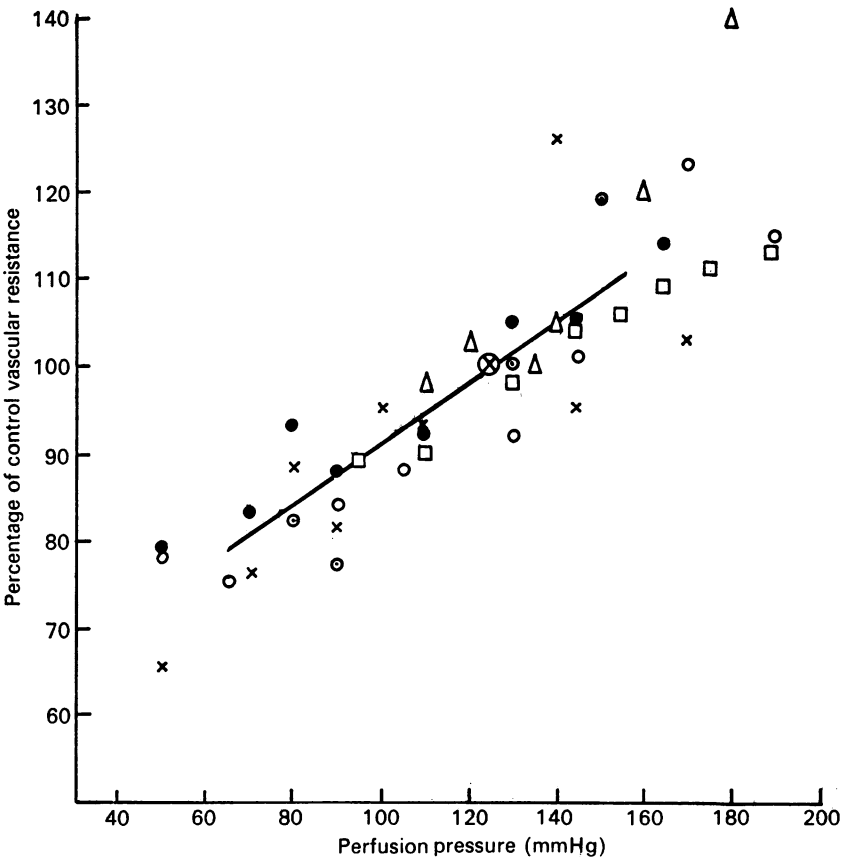


Fig. 6. The variation of renal vascular resistance with perfusion pressure in kidneys perfused with the albumin-containing medium (the different symbols represent data from six separate experiments. ⊗, control vascular resistance. The line is the best fit drawn by eye).

reticulum (Baudouin-Legros & Meyer, 1973). Angiotensin II remains extracellular while causing smooth muscle contraction (Richardson & Beaulnes, 1971). Epinephrine potentiates the coupling of excitation to contraction (Somlyo & Somlyo, 1969). Barium ions penetrate the cell membrane and have a similar action to calcium ions in causing muscular contraction. In preparations perfused with both media, the vascular contractile responses to barium ions were identical and maximal, showing that the effector organ was functional in both preparations. The responses to epinephrine and angiotensin II were markedly lower in prepara-

tions perfused with the protein-free medium. Considering the known action of the two drugs on smooth muscle, it is likely that the processes involved in the reception of a stimulus at the cell plasma membrane were affected by perfusion with the protein-free medium. It has been shown previously (Wurzel, Bacon, Kalt & Zweifach, 1964) that the sensitivity of isolated blood vessel preparations both to vasoconstrictor drugs and to an increase in tension is enhanced by the addition of plasma to the bathing solution, so that it is possible that both types of stimulation

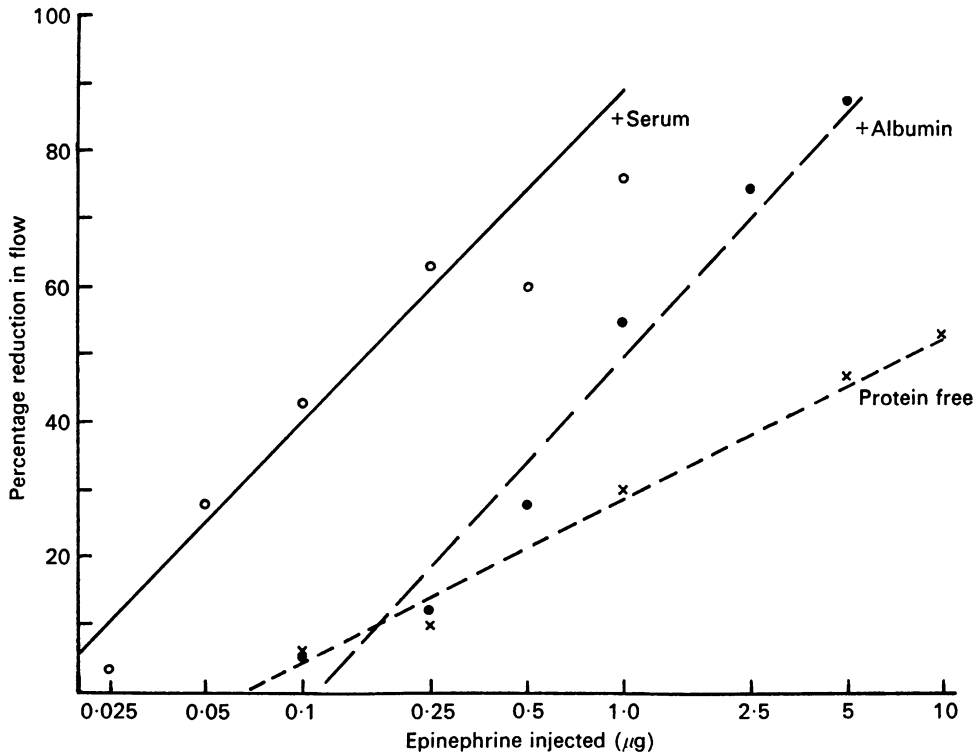


Fig. 7. The vasoconstrictor response (indicated by percentage reduction in venous outflow) to graded doses of epinephrine in kidneys perfused with the three different media for at least 20 min before testing (each point is the mean value of data for three kidneys. The lines are the best fit drawn by eye.)

are linked to events occurring at the cell plasma membrane. Since vascular muscle in kidneys perfused with the protein-free medium has a lowered sensitivity to epinephrine and angiotensin II it may also be less sensitive to changes in wall tension. It might be this effect rather than the lack of a substrate for renin that was responsible for the inability to autoregulate plasma flow in these preparations.

This hypothesis was tested using kidneys perfused with a medium containing serum albumin. The presence of serum albumin increases the sensitivity of isolated blood vessels to vasoconstrictor drugs (Wurzel *et al.* 1964), as does the presence of serum proteins. Angiotensinogen, an $\alpha_2$-globulin (Plentl, Page & Davis, 1943), is absent from a medium containing serum albumin alone. In isolated kidneys perfused with the albumin-containing medium the vasoconstrictor responses to angiotensin II

and epinephrine were enhanced, and changes in perfusion pressure resulted in changes in renal resistance. The experiments therefore produced an isolated perfused kidney preparation with the ability to autoregulate plasma flow in the absence of an extra-renal supply of angiotensinogen.

The mode of action of albumin and other proteins in maintaining the sensitivity of smooth muscle cells to constrictor stimuli is unknown. Presumably continued perfusion with a protein-free solution rapidly removes or changes the surface protein coat on the cells. This could alter the surface charge and might affect the movement of molecules through and in the plane of the cell membrane. The presence of any

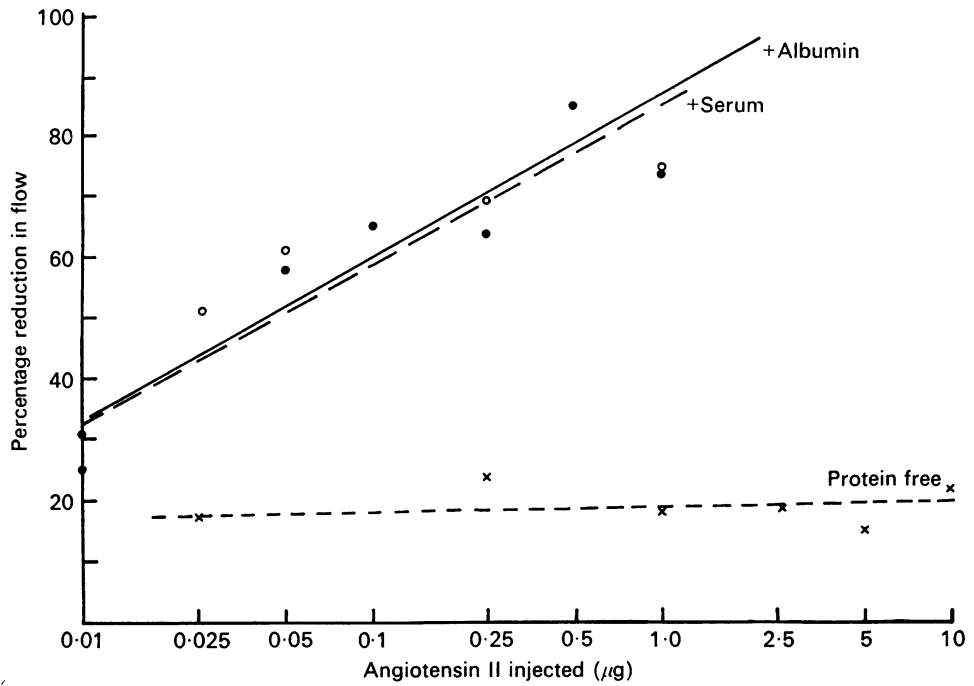


Fig. 8. The vasoconstrictor response (indicated by percentage reduction in venous outflow) to graded doses of angiotensin II by kidneys perfused with the three different media for at least 20 min before testing (each point is the mean value of data from three kidneys. The lines are the best fit drawn by eye).

suitable protein in the perfusate would reduce the leaching of protein from the cell surface. It may even be that the presence of specific proteins in the extracellular environment is required for the maintenance of membrane function.

The role of angiotensin II in autoregulation of plasma flow has been questioned in preparations other than the isolated kidney perfused with an artificial medium. Autoregulation is possible in the presence of an infusion of angiotensin II (Belleau & Earley, 1967) and in the presence of the angiotensin II inhibitor, 1-sarcosine-8-alanine angiotensin II (Anderson, Taher, Cronin, McDonald & Schrier, 1975; Kaloyanides & DiBona, 1976).

Although it is possible for autoregulation of flow to occur by a myogenic mechanism in the perfused kidney, the regulation of the renal circulation *in vivo* which also

controls the distribution of blood flow to various regions of the kidney, and whole kidney and single nephron glomerular filtration rate, almost certainly involves other than solely myogenic mechanisms.

In conclusion, the results of these experiments on the perfused kidney support a myogenic mechanism for the autoregulation of plasma flow as effected by the preparation when perfused with media containing plasma proteins. The extent to which plasma flow is autoregulated is comparable in the perfused and intact rat kidneys (Arendshorst *et al.* 1975). It has been shown that the ability of the kidney to autoregulate plasma flow with changes in perfusion pressure is not dependent on the presence of angiotensinogen in the perfusion medium. Failure of the kidney to autoregulate flow when perfused with the protein-free medium is attributed to a rapid decline in the sensitivity of the renal blood vessels to vasoconstrictor stimuli. Prevention of this decline in vascular sensitivity to constrictor stimuli maintains the ability to autoregulate plasma flow, even in the absence of extrarenal angiotensinogen.

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