Cause of Genuine Autoregulation of the Renal Circulation

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It has long been known that one of the most striking features of the normal renal circulation is its autonomous capacity to change its resistance in the face of changed arterial pressure, so that renal blood flow stays relatively constant.1, 2 Ever since, there has been much question as to whether this renal circulatory autoregulation is caused by a vital vasomotor response or by a passive physical mechanism.3 The strongest evidence that renal autoregulation may involve a mechanical response rather than one of active vasomotion is that of Winton,4, 5 who reported autoregulation of blood flow in isolated kidneys cooled to 3 to 12°C. and in kidneys poisoned with chloral hydrate. Recent work by Hinshaw and associates6, 7 and by Scher8 supports this theory, for their data suggest that the changed resistance underlying autoregulation is caused by compression of vascular channels by altered interstitial pressure. Kinter and Pappenheimer9 also believed that renal autoregulation was a passive physical phenomenon but attributed it to increased viscous resistance of cell-rich blood due to intrarenal plasma skimming. They, as well as others,10, 11 did not find autoregulation in kidneys perfused with cell-free colloidal solutions.

On the other hand, autoregulation which was not caused by interstitial pressure but was abolished by cyanide and by procaine treatment has been observed, respectively, in the in situ kidney12 and in the isolated kidney.13 Furthermore, renal autoregulation which could not be attributed to mechanical effects has also been demonstrated with cell-free colloidal perfusates.13-15 Nevertheless, Hinshaw7 recently described renal autoregulation of colloid flow apparently due to the mechanical effects of interstitial pressure.

Controversy also exists amongst the proponents of the active vasomotor theory for renal autoregulation. Page and McCubbin16 suggest that vasoconstriction mediated through an intrarenal neural plexus is responsible, since they demonstrated intrarenal autonomic ganglia, probably persisting even after chronic surgical denervation of the kidney. Sellwood and Verney17 feel that the reaction underlying autoregulation may arise from reflexogenic baroreceptors within Bowman’s capsular membrane, while the work of de Muylde18 suggests that autoregulation may be mediated by a reflexogenic function of the juxtaglomerular apparatus or the macula densa. Lamport19 champions a neurogenic conception for renal circulatory autoregulation, although objective physiologic support for a specialized receptor-neurogenic hypothesis rests largely upon the occasionally impaired autoregulation found in denervated kidneys by Brull and his collaborators.20, 21 Certain other workers14, 22, 23 have furnished evidence supporting Waugh’s postulate that autoregulation is due to a myogenic reaction of altered vasotonus in response to the level of transmural vascular pressure.13 Autoregulation of renal flow has also been attributed to removal of tissue metabolites or vasodilator material at higher pressures and flows.14, 24

The literature thus appears to be stultified with various experimental findings and opinions regarding renal circulatory autoregulation. Indeed, in a very recent investigation, Guyton and associates25 were unable to
demonstrate autoregulation of renal blood flow and they conclude that the normal kidney does not manifest this phenomenon.

The purpose of this paper is to reconcile many of the findings of various investigators and to uncover the genuine cause of autoregulation in the normal mammalian kidney.

Methods

Kidneys were excised retroperitoneally from 5 to 10 Kg. dogs anesthetized with pentobarbital a number of minutes after the kidneys had been freed of all attachments except for the renal artery and vein. Single kidneys were then placed on a mesh platform. The main renal artery was connected to a siliconized T-cannula of approximately the same bore as that of the artery and of plastic inflow tubing leading to the cannula. Two to 4 minutes after excision of the kidney, nearly or completely pulseless* perfusion was commenced, either with whole blood directly from an anesthetized dog or with a cell-free colloidal solution from a pressurized reservoir. After perfusion had been maintained for several minutes at an arterial pressure of 85 to 115 mm. Hg, initial control pressure and flow measurements were made. The renal arterial pressure was then increased over a period of 1 second or less to a pressure of 150 to 200 mm. Hg. The high pressure was maintained for ½ to 4 minutes and the pressure was then reduced to approximately the initial level. A Statham strain-gage pressure transducer connected to the side arm of the arterial cannula continuously recorded renal arterial pressure. The deep intrarenal venous pressure was simultaneously recorded by strain-gage pressure transducer connected to the side arm of the renal venous tree, and then withdrawn 2 to 5 mm. or 1.0 mm. external diameter inserted far up the renal venous tree, and then withdrawn 2 to 5 mm. to prevent its upstream tip from becoming wedged. The catheter tip (determined by renal section later) nearly always lay in an arcuate vein or, higher upstream, in an interlobular vein. A Shipley-Wilson rotameter was interposed between the perfusion source and the renal artery and/or connected to the main renal vein to record inflow or outflow or both. In all cases, the main renal venous pressure was maintained at (or less than) 2 mm. Hg above atmospheric pressure. Flow was always also measured by timed collection of 10 to 15 ml. of venous outflow before, during, and after each period with an elevated renal arterial pressure. In some experiments, the platform on which the kidney lay was suspended from the probe of a displacement transducer* so that a continuous record of kidney weight was obtained. In some experiments, the renal tissue pressure was also measured by a modification of the method of Miles and de Wardener. A 24-gauge hypodermic needle with its tip closed and 4 small holes in the terminal part of its shaft was connected by rigid tubing containing heparinized saline to a Statham strain-gage pressure transducer which measured renal tissue pressure serially after the needle tip was placed within the kidney substance. The rotameter and all strain-gage transducers were connected to appropriate amplifiers in a multichannel oscillographic recorder. A small side arm just upstream from the renal arterial cannula was used to inject 0.1 ml. volumes of test drugs into the renal artery. Renal perfusion was performed, either with whole blood directly from an anesthetized dog, or with a cell-free colloidal solution from a reservoir without recirculation.

Kidneys Perfused with Whole Blood

Anesthetized and heparinized dogs were elevated 1-2 M. above the platform on which lay the isolated kidney excised from another dog. Blood was conducted from both common carotid arteries through plastic tubing to a siliconized damping chamber and hence to a "heat exchanger." The latter consisted of a Plexiglass water-jacket housing 5 parallel siliconized brass tubes (48 cm. long and 4 mm. I.D.) through which the blood passed from and to conical terminal adapters. Water at 38 C. or 2 C. was pumped in a countercurrent manner through this water-jacket so that the blood going to the kidney was at a normal or ice-cold temperature. The blood was conducted from the heat exchanger through a small air trap into the renal arterial cannula and the temperature of the blood perfusing the kidney was monitored by a needle thermocouple placed intravascularly at the main renal vein. By rapidly releasing a screw clamp placed on the arterial conduit between the damping chamber and the heat exchanger, the nearly pulseless renal arterial pressure was rapidly raised (in 1 second) from a normal level to 150 to 190 mm. Hg. In these experiments, the main renal vein was always cannulated and the venous outflow passed through the rotameter to enter a venous collecting funnel. The blood was then returned by a Sigma-motor pump up into an external jugular vein of the blood donor dog. The venous return also contained a heat exchanger, by which the blood returned.
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ing to the dog was always maintained between 37 and 39 °C. At the beginning of each dog experiment, the pump and return system of tubing were filled with 6 per cent dextran in 0.9 per cent NaCl and the dog infused with sufficient dextran to replace the blood used to fill the arterial system of tubing (100 ml.) The Sigmamotor pump was regulated to keep approximately constant the blood volume of the dog.

Kidneys Perfused with Noncorpuscular Fluids

The basal perfusion fluid consisted of heparinized 4 Gm. per cent polyvinylpyrrolidone (PVP)-Locke solution* with its NaCl concentration reduced from 0.9 to 0.8 Gm. per cent. This colloidal solution was usually modified by the addition of fresh heparinized dog plasma to a 5 or 20 volume per cent concentration. In some experiments, the perfusate was further modified by the inclusion of chloral hydrate, yohimbine hydrochloride, proeaine hydrochloride, or y-amino-butyric acid. The pH of the perfusion fluid, determined by a pH electrometer, was adjusted to 7.25 to 7.35 before use.

The perfusate, after filtering, was added to a large reservoir kept at 39 °C. The reservoir was so pressurized by compressed gas (either 100 per cent oxygen or helium) that the tension of oxygen or helium dissolved in the perfusion liquid was maintained at approximately 960 mm. Hg. The conduit between the reservoir and the renal cannula included 2 short parallel pathways. One of the pathways was partially occluded by a screw clamp to maintain a renal arterial pressure of 85 to 115 mm. Hg, while the other was completely closed by a spring clamp. Removal of the spring clamp resulted in a rapid (less than 1 second) arterial pressure increment of about 50 to 80 mm. Hg.

Results

Whole Blood Perfusion of the Normothermic and Cold Kidney

In explanted kidneys perfused with blood at 37 to 38 °C, a sudden increase in arterial pressure produced an essentially proportional or slightly more than proportional increase in renal blood outflow during the first 2 seconds of raised pressure. In this interval, kidney weight rose rapidly (seemingly due to vascular filling) and the intrarenal venous pressure rose in parallel. Figure 1, left, records a typical experiment. Within the next 2 to 4 seconds, despite a maintained high arterial pressure, renal flow rapidly declined from its peak value (where resistance was little changed) to a minimum level, while intrarenal venous pressure transiently fell rather than rose. This progressive reduction in flow resulted from an increasing segmental resistance upstream from the deep intrarenal veins (i.e., "prevenous" resistance, calculated by dividing arterial-interlobular, or arcuate, venous pressure gradient by the rate of renal flow). Over the next few seconds, there usually followed a mild phasic augmentation of flow, due to a momentary waning of this "prevenous" resistance before an essentially steady state of flow with autoregulation was reached. Over the next half minute or more, intrarenal venous pressure and kidney weight rose gradually (presumably due to increased capillary filtration and tubular distention) with little or no further change in blood flow.

*Gratitude is expressed to Antara Chemicals, New York, N.Y., for a generous supply of PVP, average molecular weight 40,000.

Circulation Research, Volume VIII, July 1960

Figure 1

Left. Effect of a rapid increase in renal arterial pressure on venous outflow, interlobular venous pressure, renal weight, total and "prevenous" resistance in a kidney perfused with whole blood at 37 °C. Using control measurements at A and C, flow increased 18 per cent per 50 per cent increase in arterial pressure at B and 17 per cent per 50 per cent increase in arterial-interlobular venous pressure gradient. Kidney weight 25 Gm. Resistances expressed in P.A.U. as (mm. Hg x min.)/ml.

Right. Effect of a rapid increase in renal arterial pressure in the same isolated kidney 20 minutes later, when cooled to 4.5 °C. Using control measurements at D and F, flow increased 31 per cent per 50 per cent increase in arterial pressure at E and 40 per cent per 50 per cent increase in arterial-interlobular venous pressure gradient.
However, this slow increase in intrarenal venous pressure amounted to only a few mm. Hg and the final intrarenal venous pressure and effluent venous resistance reached at elevated arterial pressure remained a minor fraction (less than 25 per cent) of the total arterial-venous pressure gradient and renal resistance.

Clearly, the altered resistance responsible for the sustained autoregulation of flow was thus upstream from the intrarenal venous system. Generalized interstitial pressure effects were not the cause of this changed resistance with arterial pressure increase and of the autoregulation for such interstitial pressure changes would have restricted flow mainly by intrarenal compression of the effluent veins with resultant increased deep venous pressure. The latter remained relatively low, and since there was appreciable renal vascular flow, the actual interstitial pressure was, a priori, at least slightly lower than the measured deep intrarenal venous pressure. In the experiment shown in figure 1, left, after a steady state was reached upon increasing arterial pressure, renal blood flow had changed 18 per cent per 50 per cent change in arterial pressure (or total renal arterial-venous pressure gradient) while the flow had changed only 17 per cent per 50 per cent change in arterial-intrarenal venous pressure gradient. Since the above described type of autoregulatory response was found normally in kidneys surgically denervated and blood perfused from the living dog at normal temperature, this reaction will hereafter be referred to as genuine renal circulatory autoregulation. Genuine autoregulation is thus accomplished by resistance changes mainly upstream from the venous bed; these cause flow to change less than proportional to a change in renal arterial-venous pressure gradient.

Cooling kidneys to 3 to 10 C. reversibly abolished the above autoregulatory reaction and also drug-induced vasomotion. The cold kidney was also visibly swollen with a tense capsule, even at normal perfusion pressures. In the cold state, a rapid increment in arterial pressure effected an essentially proportionate increase in blood flow which tended to persist during continued high pressure perfusion; the rapid secondary reduction of flow found at 37 to 38 C. was absent (fig. 1, right). However, over the first half minute of raised arterial pressure, there slowly developed a marked rise in intrarenal venous pressure, a small increase in kidney weight, and a gradual reduction in flow. Renal blood flow in figure 1, right, was now changed only 31 per cent per 50 per cent increase in arterial pressure, while flow increased 40 per cent per 50 per cent increase in "prevenous" pressure gradient. This gradual flow reduction at elevated arterial pressure was thus mainly the result of abnormally high interstitial pressure, because the decreasing blood flow was mostly accounted for by a marked rise in the effluent venous resistance of the kidney at high arterial pressures during the associated cold diuresis. The resulting "autoregulation" of renal blood flow therefore appeared to be factitious, i.e., an artefact produced by abnormal changes in interstitial pressure. The slight rise in segmental resistance upstream from the deep intrarenal veins was likely due to the anomalous viscosity of blood in the postglomerular arterioles as arterial pressure and glomerular filtration rose with impaired tubular reabsorption of filtrate at such low temperatures.

In the normothermic kidney intense vasoconstriction resulted from intra-arterial injections of 1 µg. epinephrine and of 20 µg. of the autonomic ganglion stimulating drug 1, 1-dimethyl-4-phenylpiperazinium iodide (DMPP). The renal response to DMPP could be blocked by prior injection of 2.5 mg. hexamethonium chloride, thus confirming the report of Page and McCubbin. These test doses of epinephrine and DMPP were completely devoid of any vasoconstrictor effect when injected into the same kidneys cooled to 3 to 10 C. Upon rewarming the blood to normothermic levels there returned renal vasomotion due to these drugs and genuine autoregulation of blood flow.
Perfusion with Plain PVP-Locke Solutions

When oxygenated 4 Gm. per cent PVP-Locke solution was used as the sole perfusion medium, the reactivity of the kidney to various vasoconstrictor stimulants was rapidly impaired. After several minutes of perfusion with this artificial solution at normal temperature, the renal vasoconstriction evoked upon intra-arterial injections of 20 µg. of DMPP and of 1 µg. of epinephrine was much less than that observed in the normothermic blood perfused kidney and flow autoregulation was also impaired. As illustrated in figure 2, the rapidly increasing renal resistance with suddenly raised arterial pressure and genuine flow autoregulation were at times not present, even within the first 6 to 7 minutes of perfusion with the PVP-Locke solution. In other experiments, renal autoregulation of flow deteriorated less rapidly but was removed with 10 to 20 minutes of perfusion. Kidney A of figure 3 shows one such experiment in which increased renal resistance at high arterial pressure was observed initially with subsequent rapid deterioration.

This deterioration of autoregulation in the colloid perfused kidney was not caused by a specific depressant effect of the PVP, for similar results were found when dextran in a 6 per cent concentration was substituted for the PVP in the colloid-Locke perfusate.

Perfusion with Oxygenated Plasma-PVP-Locke Solutions

When heparinized dog plasma was added to the 4 Gm. per cent PVP-Locke perfusion fluids, so that the final plasma concentration was 20 per cent in the perfusate, the above described deterioration of genuine autoregulation of colloid flow with length of perfusion was prevented (fig. 3). The bona fide autoregulation with use of this perfusate was generally as intense and was as rapidly elicited as the autoregulation in the isolated whole blood perfused kidney. The reactivity of the kidney to test doses of epinephrine and DMPP was also of similar intensity.

In the typical experiment shown in figure 4, both renal arterial inflow and outflow through the main renal vein were continuously recorded along with the intrarenal venous pressure and the needle tissue pressure. Upon suddenly raising arterial pressure renal inflow and outflow, arcuate venous pressure and needle pressure rose for the first 1 to 2 seconds. There immediately followed parallel reductions in organ inflow and outflow, intrarenal venous pressure, and needle tissue pressure because of increasing vascular resistance upstream from the intrarenal veins, first manifest within 2 seconds and virtually complete within 5 seconds of raised arterial pressure. A brief waxing and waning of this upstream segmental resistance caused rhythmic changes in renal flow, intrarenal venous pressure, and needle tissue pressure because of increasing vascular resistance upstream from the intrarenal veins, first manifest within 2 seconds and virtually complete within 5 seconds of raised arterial pressure.
Deterioration of renal autoregulation with artificial colloidal perfusion and its prevention by the addition of plasma to the perfusate. Kidney A was perfused with oxygenated 4 Gm. per cent PVP-Locke solution and the plotted pressure-flow values indicate that resistance to flow at high arterial pressures declined rapidly. At 5 to 9 minutes of perfusion, renal flow rose 30 per cent per 50 per cent increment in arterial pressure. With 14 to 17 minutes of perfusion, an increase in arterial pressure caused a slightly more than proportionate change in flow and autoregulation was therefore completely removed. The 2 pressure-flow diagrams for kidney B illustrate that autoregulation did not similarly deteriorate upon perfusion with PVP-Locke solution when plasma was added to a 20 per cent concentration. Essentially the same intense autoregulation was found at 10 to 20 minutes as at 14 to 17 minutes of perfusion for flow changed 12 and 13 per cent, respectively, per 50 per cent change in arterial pressure. Ordinate, renal flow (ml. Gm./min.); abscissa, arterial pressure (mm. Hg.)

Effect of Chloral Hydrate Treatment

Both the transitory phasic flow changes upon rapidly changing arterial pressure and genuine renal autoregulation were completely abolished in every one of 4 experiments in which chloral hydrate was added to a 0.5 Gm./100 ml. concentration in the oxygenated 20 per cent plasma—80 per cent PVP-Locke perfusate. Figure 5 illustrates one such experiment. During the first 2 to 3 seconds of rapidly raised arterial pressure renal flow, arcuate venous pressure and kidney weight rose rapidly. Flow increased proportionately more than the perfusion pressure increased because of a reduction in the resistance upstream from the veins. This increase in flow was maintained while arterial pressure was kept elevated and the intrarenal venous pressure did not appreciably change further. However, renal weight rose slightly during this interval. In this experiment, flow changed 56 per cent per 50 per cent change in arterial measure, equal to the arterial-intrarenal venous pressure gradient. Thus, when tissue pressure rose to increase the eflux venous resistance and the intrarenal venous pressure, there occurred an active vasodilation in the high resistance vessels due to lessened upstream transmural pressure. The active reduction in "prevenous" segmental resistance was usually equal to the increase in venous resistance and thus flow was kept constant.

Four times more dilute perfusate concentrations of plasma (5 volumes plasma to 95 volumes PVP-Locke) only partly prevented the deterioration of genuine autoregulation of renal flow of synthetic colloidal Locke solution when oxygenated perfusion was prolonged for more than 5 to 15 minutes. However, the vaso tonic supporting or potentiating action of plasma was well manifested by the fact that the intense vasoconstrictor responses to epinephrine and to DMPP injections were maintained by 5 per cent concentrations of plasma, even though very little basal vascular tone was present upon renal perfusion with colloid containing but 5 per cent plasma.
pressure and 80 per cent per 50 per cent change in arterial-intrarenal venous pressure gradient.

In the other 3 kidneys, similarly perfused with the plasma-PVP-Locke solution containing 0.5 Gm. per cent chlortal hydrate, there resulted a slow reduction in the augmented renal flow and a progressively more marked rise in intrarenal venous pressure and effluent venous resistance when arterial pressure was maintained elevated for the same length of time. This factitious or pseudo-autoregulatory reaction resembled that seen in the kidney cooled to 3 to 10 C. in that it was also produced by excessive increases in extravascular pressure at elevated arterial pressures. In these 3 chlortal hydrate treated kidneys, renal flow rose 35, 36, and 41 per cent per 50 per cent increase in arterial pressure, while renal flow changed 52, 50 and 60 per cent, respectively, per 50 per cent change in upstream pressure gradient (arterial-intrarenal venous pressure gradient).

Chlortal hydrate markedly dilated the high resistance vessels of the kidney in every case, as the renal flows ranged between 5.5 and 7.7 ml./min. per Gm. of kidney weight at arterial pressures of 100 mm. Hg and the intrarenal venous pressures were unusually high (30 to 42 mm. Hg). The chlortal hydrate treatment made the renal vessels totally non-reactive to many stimuli for, besides abolishing the basal vascular tone of the kidney and genuine autoregulation, vasoconstriction was not elicited upon injecting either 1 μg. of epinephrine or 20 μg. of DMPP into the renal artery.

Effect of Gamma-Aminobutyric Acid Treatment

γ-aminobutyric acid (GABA) is a compound which blocks sensory discharges of slowly adapting neural stretch receptors of crustacean skeletal muscle. Recent evidence by Stanton and Woodhouse, although interpreted otherwise, suggests that GABA may inhibit the slowly adapting mechanoreceptors in the carotid sinus and aortic areas of the dog. It therefore was of interest to determine whether renal circulatory autoregulation might be inhibited by GABA. The rapid phasic alterations in renal flow, deep venous pressure, and needle tissue pressure seen in normal kidneys within the first few seconds in response to a sudden change in arterial pressure and the steady state autoregulation of the normal kidney were not at all affected by GABA in perfusate concentrations as high as 0.5 Gm./100 ml. Figure 6 records one such experiment in which the isolated kidney was perfused with oxygenated 20 per cent plasma—80 per cent PVP-Locke solution. When a constant flow was reached in 15 seconds of elevated arterial pressure, flow had increased 12 per cent per 50 per cent increase in arterial pressure and 11 per cent per 50 per cent increase in arterial-arcuate venous pressure gradient. In the calculations of total and “prevenous” resistance, the values for venous outflow were used.
Effect of a rapid increase in renal arterial pressure on arterial inflow, arcuate venous pressure, and renal weight and on total and "prevenous" resistance in a kidney treated with chloral hydrate. Using control measurements at A and C, flow increased 56 per cent in 50 per cent increase in arterial pressure at B and 80 per cent per 50 per cent increase in arterial arcuate-venous pressure gradient. Data at 17 to 18 minutes of perfusion with oxygenated 20 per cent plasma—80 per cent PVP-Locke solution containing 0.5 Gm. per cent chloral hydrate. Kidney weight 22 Gm.

resistance response reached its peak in 4 to 5 seconds. At this peak of the increased resistance response to elevated pressure, renal flow was even slightly less than the steady state flow rate at the lower perfusion pressure. This reaction time (also clearly demonstrated in fig. 4 when no GABA was used) was seemingly too fast for autoregulation to be explicable in terms of changes in tissue metabolites and was of the same order of magnitude as the reaction time for smooth muscle contraction following direct or nervous stimulation.34, 35 Furthermore, the intrarenal venous pressure rose very little as flow was maintained constant at the elevated arterial pressure.

Upon rapidly lowering the elevated arterial pressure, there was found within the first few seconds of lowered arterial pressure an even greater upstream resistance, soon replaced by lowered resistance due to active vasodilation upstream from the veins. Similar findings are recorded in figures 1, 7 and 8.

It therefore appears that the physiologic autoregulatory reaction of the kidney is accomplished by smooth muscle contraction or augmented vasotonus in response to tension, deformation, or stretch. However, it inductively appears from the failure of inhibition of autoregulation by GABA that this flow autoregulation may not result from intrinsic reflexes arising from stimulation of dendritic mechanoreceptors.

Effect of Adrenergic Blocking Agents

Dibenzyline (phenoxybenzamine hydrochloride) is both an irreversible competitive adrenergic and histaminergic antagonist blocking sympathetically induced vasoconstriction. Intrarenal sympatholysis of the surgically denervated (excised) kidney was thus achieved by intravenous administration of 20 mg. of Dibenzyline per Kg. body weight to the intact dog 2 hours before excision and isolated perfusion of the kidney. Sympatholysis was assuredly complete with Dibenzyline within the kidney perfused with oxygenated 20 per cent plasma solutions for this drug completely abolished the vasoconstriction arising from intra-arterial injections of 20 μg. DMPP and of 1 μg. epinephrine. Upon sudden increments in arterial pressure, there were observed rapid phasic alterations in renal flow, intrarenal venous pressure, kidney weight and upstream segmental resistance (fig. 7). The degree of genuine autoregulation was marked but less than perfect, renal flow rising 22 per cent per 50 per cent increase in arterial pressure in the autoregulatory state shown in figure 7.

A noteworthy difference in kidneys denervated by Dibenzyline was that the amplitude of the rapid flow increase within the first 1 to 2 seconds of suddenly raised arterial pressure was much greater than that normally seen in the kidney perfused either with whole blood or with oxygenated 20 per cent plasma solutions (fig. 7 with fig. 1, 4, and 6). It was due to a much greater rapid distensi-
bility of the high resistance vessels following the administration of the adrenergic blocking drug. That it was not the result of a specific effect of intrarenal denervation on reaction time appears to follow from experiments with yohimbine, with anoxic perfusion and with procaine treatment (see below).

Yohimbine, a reversibly competitive adrenergic blocking drug, and also a specific antagonist of serotonin and a local anesthetic, was added to the oxygenated 20 per cent plasma—80 per cent PVP-Locke solution. Perfusion was performed without antecedent administration of a blocking agent to the kidney donor dog. Intrarenal sympatholysis produced by a yohimbine concentration of 400 µg./100 ml. perfusate did not impair genuine autoregulation of renal flow. In fact, in the yohimbine experiment shown in figure 8, marked rhythmical variations in flow and "prevenous" vascular resistance occurred during the first 30 seconds of elevated arterial pressure wherein, at the fourth second of elevated pressure, flow paradoxically fell to a fraction of the flow rate found at the much lower preceding control pressure. In the ensuing steady state of autoregulation, renal flow changed only 11 per cent relative to a 50 per cent increase in arterial pressure and 10 per cent per 50 per cent change in "prevenous" pressure gradient.

Such yohimbine concentrations of 400 µg./100 ml. completely blocked the vasoconstrictor response normally evoked by 20µg. of DMPP injected intra-arterially and 1 µg. injections of epinephrine induced negligible vasoconstriction. Yohimbine was much less effective in blocking renal vasomotor responses to intra-arterial injections of serotonin creatinine sulfate (1-5 µg.), as has been found with other adrenolytic agents.16

Effect of Anoxic Perfusion

With 10 to 20 minutes of anoxic perfusion with 20 per cent plasma—80 per cent PVP-Locke solution (produced by substituting helium for oxygen in the perfusate), the changes found upon suddenly raising renal arterial pressure were similar to those observed in the kidney treated with Dibenzyline to complete sympatholysis. In particular, the amplitude of the flow increase in the first 1 to 2 seconds of raised arterial pressure was much greater than that normally found and flow autoregulation was impaired, e.g., flow increased 25 per cent per 50 per cent increase in arterial pressure and 28 per cent per 50 per cent increase in arterial-arcuate venous pressure gradient.

However, even after 30 minutes of anoxic perfusion, intra-arterial injection of 20 µg. DMPP elicited intense renal vasoconstriction. Therefore, the disproportionate amplitude of the flow increase within the first 2 seconds of raised arterial pressure and the moderate impairment of bona fide autoregulation with a few minutes of anoxic perfusion, seemed to result directly from anoxic depression of the reactivity of vascular smooth muscle to pressure rather than from anoxic ganglionic depression.
Effect of a rapid increase in renal arterial pressure on renal weight, arcuate venous pressure, and arterial inflow and also on total and "prevenous" resistance in a kidney treated with Dibenzyline to complete sympatholysis. Using control measurements at A and C, flow increased 22 per cent per 50 per cent increase in arterial pressure at B and 20 per cent per 50 per cent increase in arterial-arcuate venous pressure gradient. Perfusate consisted of oxygenated 20 per cent plasma—80 per cent PVP-Locke solution devoid of Dibenzyline. Kidney weight 26.5 Gm.

Effect of Procaine Treatment

With a procaine concentration of 500 µg./100 ml. of perfusate, anesthetization of intrarenal nervous elements was achieved without appreciable depression of vascular smooth muscle responses to direct stimuli. Such procaine treatment of the kidney perfused with oxygenated 20 per cent plasma—80 per cent PVP-Locke solution completely prevented the vasoconstrictor response neurogenically evoked following intra-arterial injections of 20 or 40 µg. of DMPP. However, this anesthetization did not appreciably lessen the renal vasoconstriction directly elicited upon intra-arterial injections of either 1 µg. of epinephrine or 2.5 mg. of barium chloride. Figure 9 shows that with procaine concentrations of 500 µg./100 ml., the amplitude of the passively induced flow increase within the first 2 seconds of raised arterial pressure was not exaggerated and that the genuine autoregulatory reaction which followed was intense. Thus, in this experiment renal vascular flow increased only 13 per cent per 50 per cent sustained increase in arterial pressure and 12 per cent per 50 per cent increase in arterial-arcuate venous pressure gradient.

Twenty times greater perfusate concentrations of procaine (0.1 Gm./100 ml.) abolished both the active changes in renal resistance normally observed within the first 10 to 30 seconds of suddenly raised arterial pressure and the flow autoregulation. Flow now changed more than proportional to changes in perfusion pressure. In the typical experiment shown in fig. 10, renal flow increased 64 per cent, both per 50 per cent increase in arterial pressure and per 50 per cent increase in pressure gradient upstream from the arcuate veins. The intense renal vasoconstriction normally evoked by intra-arterial injections of 1 µg. epinephrine and 2.5 mg. of barium chloride were largely prevented by this higher concentration of procaine.

It is therefore evident from the above experiments that procaine in amounts necessary for nerve anesthetization or nerve blockade of the kidney does not appreciably affect autoregulation, but that much larger doses of procaine depress directly and markedly the reactivity of the vascular smooth muscle cells to various direct stimulants. These stimulants include epinephrine, the barium ion, and the stimulus responsible for normal autoregulation of renal flow. The loss of renal autoregulation previously reported by Ochwald,38 by Wangh,13 and by Weiss, Passow and Rothstein,15 with procaine concentrations of 0.1 Gm. per cent, or higher, was therefore not due to its effect on an intrarenal neural or neurogenic mechanism but due to its direct depressant effect on vascular smooth muscle.

Effect of Hemorrhage

Severe hemorrhage in the dog before excision of the kidney resulted in intense renal vasoconstriction persisting after excision and isolated perfusion of the kidney with oxygenated 20 per cent plasma—80 per cent PVP-Locke solution. Within the first fifteen minutes of perfusion, the rate of renal colloid flow was very low (about 0.7 ml./min./Gm. kidney weight) at arterial pressures of 100 mm. Hg and a sudden increase in arterial pressure and 12 per cent per 50 per cent increase in arterial-arcuate venous pressure gradient.
pressure did not even transiently distend the high resistance vessels, for there was a very large resistance upstream from the interlobular veins which remained constant upon raising pressure. Phasic changes in resistance and flow, normally present during the first few seconds of raised pressure, were absent and genuine autoregulation of flow was not found. The high resistance vessels of the kidney thus appeared to be in a spastic, relatively nondistensible and nonreactive state of smooth muscle contracture or rigor.

As isolated perfusion was further continued, the vascular contracture lessened and renal vascular flow rose gradually. After 20 minutes of perfusion, slight phasic changes in resistance and flow were present within the first 5 to 10 seconds of raised arterial pressure and mild genuine autoregulation of flow was demonstrated. After 30 minutes of colloidal perfusion, when renal flow was yet subnormal but further increased to about 1.3 ml./min./Gm. kidney weight, distensibility had evidently returned to the high resistance vessels, for the resistance upstream from the interlobular veins now fell slightly with the first 1 to 3 seconds of suddenly elevated arterial pressure before augmented vasoconstriction manifested itself. The active autoregulatory reaction was now quite intense because renal flow increased only 22 per cent per 50 per cent sustained increase in arterial pressure and 21 per cent per 50 per cent rise in arterial-interlobular venous pressure gradient.

De Wardener and Miles,39 and Ritter,27 have observed that circulatory autoregulation was impaired or abolished in the kidney in situ, when hemorrhage or prolonged experimental procedures induced sustained renal vasoconstriction. Their impairment or loss of autoregulation appeared greater than could be attributed to a mere masking of autoregulation by increased segmental resistance in series with the resistance mechanism responsible for autoregulation. However, the actual cause of the loss of autoregulation was not clear. Our experiments suggest that with sufficiently prolonged intense renal vasoconstriction secondary to hemorrhage, there results vascular smooth muscle contracture in which the renal blood vessels are relatively nonreactive to distending pressure. This rigor *intra* vitam seems to reside largely within the high resistance vessels, normally responsible for the autoregulation of the renal circulation.

**Discussion**

This investigation has apparently resolved the question as to whether autoregulation of renal blood flow in the normal kidney is due to a passive or active mechanism in favor of the latter. That it is not caused by the viscous effect of blood cells circulating through the kidney is certain, for intense and at times essentially perfect autoregulation in the complete absence of red blood cells has been again demonstrated, confirming work done in various laboratories.13-15 The finding that renal autoregulation deteriorates rapidly with artificial colloidal perfusates and that this deterioration is prevented by the inclusion of...
Effect of a rapid increase in renal arterial pressure on arcuate venous pressure and arterial inflow and on total and "prevenous" resistance in a kidney treated with procaine to nerve anesthetization without appreciable depression of vascular smooth muscle. When a constant flow was reached in 18 seconds of elevated arterial pressure, flow had increased 13 per cent per 50 per cent increase in arterial pressure and 12 per cent per 50 per cent increase in arterial-arcuate venous pressure gradient. Data at 19 to 20 minutes of perfusion with oxygenated 20 per cent plasma—80 per cent PVP-Locke solution containing 500 μg per cent procaine. Kidney weight 28 Gm.

Figure 9

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blood plasma in the perfusate seems to explain the results of Kinter and Pappenheimer, which they attributed to their cell separation theory. Kintner and Pappenheimer found that the addition of red blood cells (unwashed) in sufficient concentrations to a synthetic dextran perfusate increased renal resistance and resulted in autoregulation, not present in their simply dextran perfused and vasodilated kidneys. Apparently, a plasma factor trapped between their red cells or a similar factor (diffusible) present within their red blood cells was responsible for the support of the basal resistance and autoregulation in their kidney experiments. The nature of this vasoconstrictive or vasoexcitatory factor (or factors) is yet unknown, but it does not seem to be an adrenergic or histaminergic-like compound because vascular reactivity and autoregulation in the plasma perfused kidney was not abolished by use of appropriate blocking drugs.

The same type of flow autoregulation, normally elicited rapidly in both the innervated intact kidney and the surgically denervated kidney in situ, we have demonstrated in excised kidneys perfused with whole blood and with 20 per cent plasma—80 per cent PVP-Locke solutions. Our recordings of intrarenal venous pressures, needle tissue pressures (usually slightly lower than the measured intrarenal venous pressure), and weight changes in the isolated kidney cannot account for this type of autoregulation on a passive physical basis and indicate that it is due to an active physiologic process. These recordings thus refute the interstitial pressure notions of Hinshaw and associates and of Scher which they applied to the normal kidney. The term genuine renal circulatory autoregulation is therefore suggested for this autonomous feature of the normal kidney to distinguish it from nonnormal or factitious mechanisms which may also cause renal vascular flow to change less than proportional to changes in perfusion pressure gradient.

The evidence of Winton that autoregulation of flow is probably caused by some passive rather than active process is no longer tenable for the normal kidney. The imperfect autoregulation, observed by Winton, which we have confirmed in kidneys cooled to 3 to 10°C, and in those treated with chloral hydrate, is due to the physical effects of abnormally high tissue pressures. This type of autoregulation is thus a spurious variant.

Our data further indicate that the resistance changes responsible for genuine autoregulation of renal blood flow lie upstream from the intrarenal vein and within the arterial-arteriolar vasculature or high resistance vessels. That the resistance changes underlying this flow autoregulation are largely
preglomerular and also responsible for autoregulation of glomerular filtration rate as arterial pressure is varied appears to follow also from the elegant work of Thurau and Kramer. 40

Three different procedures, rendering the renal vasculature quite inert, further demonstrate that active changes in vasomotion cause the resistance changes responsible for genuine autoregulation of renal flow. Cooling kidneys to 3 to 10 C., treating kidneys with 0.5 Gm. per cent concentrations of chloral hydrate, and the use of procaine concentrations of 0.1 Gm. per cent, abolished both active vasomotion in response to drugs and the autoregulation of the normal kidney. Three other procedures have been previously shown to remove renal autoregulation: oil perfusion, 13, 41 cyanide treatment12, 35, 42 and papaverine administration.10

The following observations indicate that changes in tissue vaso-active metabolites are not responsible for this circulatory autoregulation of the normal kidney. Anoxic perfusion did not soon abolish the genuine autoregulatory reaction. The active changes in the high resistance vessels responsible for autoregulation first became manifest in the second to fifth second of suddenly raised arterial pressure, a reaction time of the same order as that for smooth muscle contraction upon direct or nervous stimulation. 34, 35 Active rhythmic changes in flow and arterial-arteriolar resistance were often apparent during the first 10 to 30 seconds of pressure elevations from normal perfusion levels. Essentially perfect flow autoregulation was occasionally found in which, once a new steady state of vascular resistance was reached, flow was practically unchanged despite a large increase in perfusion pressure gradient.

We have also demonstrated that genuine autoregulation of renal flow is not caused by a basic neurogenic reaction mediated through either axon reflexes or interneuronally. This autoregulation in the isolated kidney was not appreciably impaired by procaine anesthetization of intrarenal neurons in a manner not concomitantly depressant to the reactivity of vascular smooth muscle cells. Furthermore, yohimbine, an adrenergic blocking agent, in concentrations causing intrarenal sympatholysis, did not depress the intense genuine autoregulation demonstrable in the completely isolated kidney. With intrarenal sympatholysis following Dibenzyline administration, autoregulation in the excised kidney was no more than slightly impaired. It is interesting that y-aminobutyric acid, which may inhibit neural stretch receptors or mechanoreceptors in the dog, did not impair renal circulatory autoregulation.

The results of Brull and associates20, 21, 43 require clarification, because they have been interpreted as favoring a neurogenic basis for renal autoregulation whereas it actually appears that the nerves of the kidney in autoregulation play at best a nonessential role of potentiation or of keeping the vascular smooth muscle cells in a normally quite re-
active state for inherent responses. Brull and associates found that with nondenervated kidneys perfused in situ, renal blood flow almost always remained essentially constant when renal arterial pressure was raised from normotensive levels to pressures as high as 220 to 270 mm Hg. However, with acute or chronic surgical denervation, or with the use of drugs presumably denervating kidneys pharmacologically, renal blood flow increased variably as arterial pressure was raised. It is most important, we believe, that perfect autoregulation (2 per cent flow change per 50 per cent arterial pressure change) was found in the same laboratory in one experiment with chronically denervated kidneys and that in many of Brull's experiments renal autoregulation was still present, although imperfectly so. Other workers have demonstrated nearly or essentially perfect autoregulation of renal flow, following, as a rule, acute or chronic surgical denervation of the kidney, for their average changes in renal flow relative to a 50 per cent change in arterial pressure were between 5 and 10 per cent. Thus, the reactivity of vascular smooth muscle itself was apparently depressed in many of the denervation experiments of the above Belgian workers.

From this investigation and from the preceding discussion, it therefore follows that intrinsic (myogenic) smooth muscle cell responses within the high resistance renal vessels are responsible for genuine autoregulation of the kidney circulation upon variations in arterial pressure (arterial-venous pressure gradient). The response is a homeostatic one of vasoconstriction or augmented vasoconstriction when the transmural vascular pressure is elevated and vasodilation or lessened vasoconstriction when the transmural pressure is reduced.

The type of blood vessel reaction responsible for renal flow autoregulation does not appear to be unique to the kidney. It has been observed by Folkow and others in nonrenal vascular beds. Further work is needed to determine whether the direct stimulus for the myogenic vasomotor reaction to the level of renal transmural vascular pressure is tension, deformation, or stretch. The paradoxical effect of a constant amount of increased renal vascular resistance (vasoconstriction) at sustained higher transmural or distending pressures may be due to the action of a tension or deformation stimulus, continuing despite a tonic reduction in vascular bore. However, the vascular paradox is more likely caused by the net effect of stretch or tension induced unsynchronized vasomotion, recurring in innumerable myogenic units within the interlobular arteries and the many thousands of preglomerular arterioles of the mammalian kidney. The rhythmic changes in renal resistance and flow observed within the first 30 seconds of suddenly raised arterial pressure, support this conception, for the sudden pressure change probably produced temporarily much more synchronous vasomotion.

No specialized vascular mechanoreceptors need be postulated at this time to explain the basic myogenic nature of renal circulatory autoregulation, since Bulbring has shown that stretch of smooth muscle cells of the taenia coli acts as a stimulus for increased myogenic automaticity and that the element of the smooth muscle cell sensory to stretch is closely combined with the properties of a tension producing element. However, specialized myocytes particularly sensitive to vascular deformation or stretch may be normally involved in renal flow autoregulation, especially when the autoregulation is essentially perfect, since this organ is unique in its huge capacity for autonomous vasomotor adjustments to changes in vascular pressure. If specialized myocytes are indeed involved, the afibrillar and paucifibrillar myocytes of the juxtaglomerular apparatus would appear to be, as suggested by the earlier work of Goormaghtigh, the smooth muscle cells which act as more excitable pacemakers, analogous to the nodal myocytes of the heart, in the myogenic automaticity and flow autoregulation of the kidney. It is submitted that the basic means of conduction of this autoregulatory response is by nonneural and nonhormonal conduction from myocyte to myocyte, probably by electrical intercell transmission.
This hypothesis that the juxtaglomerular apparatus subserves the myogenic vasemotion responsible for renal circulatory autoregulation may account for the fact that there is no appreciable renal autoregulation of the small fraction of blood flowing through the medullary blood vessels. The juxtaglomerular apparatus is said to be normally less developed in the juxtamedullary region of the cortex. Langston, Guyton and Gillespie were unable to find renal circulatory autoregulation upon varying renal arterial pressure in laparotomized and aortic cannulated dogs. Since autoregulation did not appear after spinal anesthesia, although the absolute rate of renal flow rose somewhat with this treatment, they concluded that their inability to demonstrate renal autoregulation was not the result of prolonged vasoconstriction due to sympathetic activity. We wish to point out, however, that their rates of renal blood flow at normal perfusion pressures were subnormal even after sympathetic blockade (less than 2 ml./min./Gm. of kidney weight). Their results are not at all contrary to the fact that the normal kidney does manifest circulatory autoregulation. Cannulation of the abdominal aorta frequently causes renal vasoconstriction and loss of autoregulation, but not with Brull's technic.

Probably relevant to the absence of autoregulation in the experiments of Langston, Guyton, and Gillespie is our finding that the renal ischemia, developing in situ with prolonged vasoconstriction following hemorrhage, is maintained for some time after acute surgical denervation and completely isolated perfusion of the kidney. In this state, the high resistance vessels of the isolated kidney were found to be relatively nondistensible to intravascular pressure and also relatively nonreactive to certain stimuli, since the genuine renal autoregulatory reaction had been reversibly removed. It is therefore suggested that this slowly reversible spastic state of renal ischemia is due to smooth muscle contracture of the high resistance vessels, a rigor in a vita, presumably due to an inextensible tight association of actin and myosin filaments intracellularly. Its actual pathogenesis is presently obscure, but it may arise similarly to the smooth muscle contracture arising elsewhere, following intense stimulation with epinephrine.

**Summary**

The mechanism causing renal vascular flow to vary less than proportionally to changes in arterial-venous pressure gradient, was studied in isolated dog kidneys perfused with whole blood and with cell-free colloidal solutions. This autoregulation of renal flow rapidly deteriorated along with vascular reactivity to drugs when oxygenated polyvinylpyrrolidone-Locke solution was used for perfusion. This deterioration was prevented by the addition of plasma to the colloidal perfusate.

During the first 2 seconds of suddenly raised arterial pressure, renal flow normally increased proportionately or slightly more than proportionately to the increase in arterial pressure; intrarenal venous pressure, needle tissue pressure and kidney weight rose simultaneously. During the next 4 seconds, increasing vascular resistance upstream from the intrarenal veins caused parallel reductions in renal flow, intrarenal venous pressure, needle tissue pressure and at times kidney weight. After brief rhythmical changes in prevenous segmental resistance, flow became steady to show intense autoregulation, while intrarenal venous pressure and needle tissue pressure remained relatively low.

This genuine autoregulation of renal flow was abolished by cooling kidneys to 3 to 10 C., and by treatment with chloral hydrate and with procaine in concentrations rendering the smooth muscle of the renal blood vessels relatively inert to direct drug stimulants. On the other hand, at temperatures of 3 to 10 C., and usually with chloral hydrate treatment, a factitious and passive type of flow autoregulation was observed, caused by the effects of abnormally high tissue pressures.

Renal flow autoregulation was not appreciably impaired by anesthetization of the in-
intrarenal nerves by procaine in concentrations which did not simultaneously depress vascular smooth muscle reactivity. Yohimbine induced sympatholysis did not impair autoregulation, and Dibenzyline treatment to intrarenal sympatholysis depressed only slightly autoregulation of renal flow. It was not inhibited by y-aminobutyric acid.

Anoxic perfusion which did not appreciably depress the reactivity of intrarenal autonomic ganglia, impaired autoregulation moderately. The loss of autoregulation of renal flow, accompanied by vasoconstriction following severe hemorrhage in the kidney donor dog, was slowly reversible upon perfusion of the subsequently isolated kidney and was related to smooth muscle contracture within the arterial-arteriolar vasculature.

It is concluded, that myogenic vasomotion in the renal arterial-arteriolar tree in response to the level of transmural vascular pressure is the fundamental cause of genuine renal circulatory autoregulation. It is furthermore suggested that the myocytes of the juxtaglomerular apparatus may act as myogenic pacemakers in the vasomotion responsible for the essentially perfect autoregulation of the normal kidney.

**Summario in Interlingua**

Le mecanismos que es responsabile pro le facto que le fluxo reno-vascular non varia a grados proomissionale al alterationes del gradiente de pression arterio-venose essava studiate in isolato ren al perfusato colloidic.

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*Circulation Research, Volume VIII, July 1960*
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Cause of Genuine Autoregulation of the Renal Circulation
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Circ Res. 1960;8:871-888
doi: 10.1161/01.RES.8.4.871

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