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ROLE OF CALCIUM AND ALBUMIN IN THE AUTOREGULATION OF RENAL PERFUSATE FLOW

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SUMMARY

1. We investigated the role of calcium and albumin on the perfusate flow in isolated perfused rat kidneys. The initial perfusion medium was Krebs-Henseleit bicarbonate solution with 1·82 mM-calcium without albumin. Perfusate flow autoregulation occurred above 100 mmHg.

2. Raising albumin concentration to 20 and 60 g/l. abolished autoregulation and increased perfusate flow.

3. Keeping ionized calcium at 1·82 mM restored autoregulation in medium containing 20 and 60 g albumin/l. However, in 60 g albumin/l. autoregulation occurred at a significantly higher flow.

4. 1·82 mM-ionized calcium appears to be a critical level for autoregulation of flow in these experiments, for autoregulation was not obtained in 60 g albumin/l. medium containing 1·80 mM-ionized calcium. On the other hand, autoregulation occurred in medium containing 1·83 mM-ionized calcium, but at a lower perfusate flow.

5. Raising albumin concentration to 120 g/l. increased perfusate flow from $14{\cdot}6 \pm 0{\cdot}8$ to $20{\cdot}8 \pm 0{\cdot}7$ ml./min.g ($n = 5$, $P < 0{\cdot}01$) in the presence of 1·82 mM-total calcium, and from $11{\cdot}6 \pm 1{\cdot}0$ to $15{\cdot}8 \pm 0{\cdot}7$ ml./min.g ($n = 5$, $P < 0{\cdot}01$) in 1·82 mM-ionized calcium. The effect of raising albumin concentration was reversible.

6. Removing the capsule from the kidneys abolished the increased flow in response to raising albumin concentration.

7. We conclude that (*a*) the mechanism for the autoregulation of renal perfusate flow in isolated perfused kidneys is critically dependent on an extremely narrow range of ionized calcium concentration in the perfusion medium, below this range, autoregulation is not achieved; above it, however, autoregulation is achieved, but during intense vasoconstriction; (*b*) raising albumin concentration in the perfusion medium increases perfusate flow and abolishes autoregulation by lowering extracellular ionized calcium and by raising intrarenal tissue pressure.

INTRODUCTION

The kidney has the remarkable ability of keeping its blood flow relatively constant over a wide range of renal perfusion pressure. Though several hypotheses have been proposed to explain this autoregulation of blood flow, there is reason to believe that

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calcium may play an important role. In 1973, Israelit and co-workers suggested that calcium delivery to the distal tubule was necessary for the autoregulatory response (Israelit, Rector & Seldin, 1973). Burke and collaborators supported this view (Burke, Navar, Clapp & Robinson, 1974). A year later, Ono and associates published data indicating that the calcium antagonists, nifedipine and verapamil, blocked renal autoregulation of blood flow (Ono, Kokubun & Hashimoto, 1974). They attached no special significance to these observations in the general context of the mechanisms of autoregulation; thus not much has been reported along the lines of calcium since the paper appeared, except a recent report by Chomdej, Bell & Navar (1977) showing that infusion of calcium drastically reduced the blood flow at which autoregulation occurred. While conducting experiments on the role of calcium in the mechanism controlling renin secretion from isolated perfused kidneys, we have observed that autoregulation of perfusate flow is dependent on a specific range of ionized calcium concentration in the perfusion medium. The aim of this paper is to report some of these findings. An additional aim of the paper is to enquire by what mechanisms raising albumin concentration in the perfusion medium abolishes autoregulation and increases perfusate flow.

METHODS

Male Sprague-Dawley rats (153–228 g) from Charles River Breeding Laboratories (Wilmington, Massachusetts) were fed a sodium-deficient rat food containing 2 m-equiv sodium/kg (Bio Serve, Frenchtown, New Jersey) and deionized water. They were maintained on this regimen for at least 1 week before the right kidney was prepared for perfusion.

The kidneys were perfused by a procedure described previously (Fray, 1976). Briefly, we anaesthetized each rat with sodium pentobarbitone (40 mg/kg I.P.) and placed it on a stand. Through an abdominal incision, we exposed, cannulated, and then removed the right kidney without interruption of flow. The kidney was transferred to a perfusion reservoir in a temperature controlled box (37 °C) and perfused with Krebs-Henseleit bicarbonate solution with or without bovine serum albumin (*Fraction V*, powder, Miles Laboratories, Eckert, Indiana). Perfusion pressure was measured by an anaeroid manometer placed directly in the perfusion system and perfusate flow measured by a timing syphon placed just beneath the kidney. The perfusion medium consisted of either 0, 20, 60, or 120 g albumin/l. The Krebs-Henseleit solution contained the following constituents: Na^+, 145 mM; K^+, 4 mM; Mg^{2+}, 1 mM; Cl^-, 128 mM; PO_4^{2-}, 1 mM, HCO_3^-, 25 mM; and varying concentrations of Ca^{2+}. Ionized Ca^{2+} was measured by a calcium-sensitive electrode (The London Co.). Calcium chloride was added to the perfusion medium without ionic substitution. Glucose was added to give a final concentration of 10 mM. The perfusion medium was prepared at least a day in advance and stored at −40 °C. Before an experiment, the medium was thawed, poured in the perfusion reservoir, gassed at 95 % O_2 and 5 % CO_2, and recirculated.

Each kidney was perfused for an initial 10 min period after which the perfusion medium was discarded. It was then switched to fresh medium identical to the first. Six kidneys were perfused without albumin but with Krebs-Henseleit solution containing 1·82 mM-calcium. These kidneys were perfused for several successive 5 min periods, first at 100, 125, and then at 150 mmHg; they were returned back to 100 mmHg, and then lowered to 75 and 50 mmHg, and finally returned back to 100 mmHg. In an additional series of experiments, kidneys were perfused for four 5 min periods during which mean perfusion pressure was raised from 80 mmHg and held constant at 100, 120, and 140 mmHg. This is a range in which blood flow autoregulation usually occurs (Shipley & Study, 1951).

In the series of experiments to determine the mechanisms by which raising albumin concentration increased flow, we perfused the kidneys for four 5 min periods at 100 mmHg. During the first, second, and third periods the perfusion medium contained 20, 60, and 120 g albumin/l. with either 1·82mM-total calcium or 1·82 mM-ionized calcium; during the fourth period the kidneys were returned to 20 g albumin/l. In a series of identical experiments, the renal capsule was peeled off before we removed the kidney from the rat.

The flow rate was averaged over each 5 min perfusion period. Statistical significance was assessed using paired t test for each experiment and unpaired t test for comparing experiments.

RESULTS

Fig. 1 illustrates two distinct effects of raising albumin concentration on perfusate flow in the isolated perfused kidney. The first is that it significantly increased perfusate flow at all perfusion pressure studied ($P < 0{\cdot}05$). The second is that raising albumin concentration abolished perfusate flow autoregulation. Without albumin, perfusate flow remained constant throughout the autoregulatory range (100–150 mmHg) and fell when pressure was lowered below this range (75–50 mmHg).

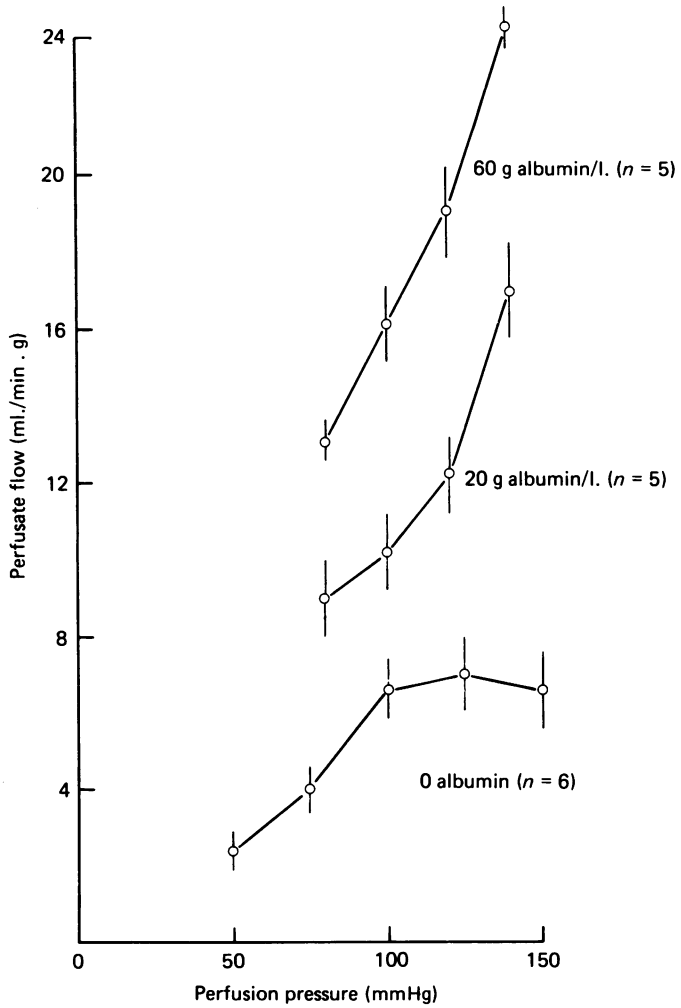

Fig. 1. Raising albumin concentration in the perfusion medium increased perfusate flow and abolished autoregulation. The total calcium was 1·83 mM in the medium without albumin and 2·5 mM in that with albumin. Each point represents mean + S.E. of mean of the perfusate flow averaged over a 5 min perfusion period.

Since raising albumin concentration abolished perfusate flow autoregulation at pressures throughout the autoregulatory range, we sought to determine the mechanism. We reasoned that raising albumin concentration might lower the ionized calcium from a level critical to the autoregulatory mechanism. Fig. 2 shows that raising albumin concentration in the perfusion medium lowered ionized calcium as

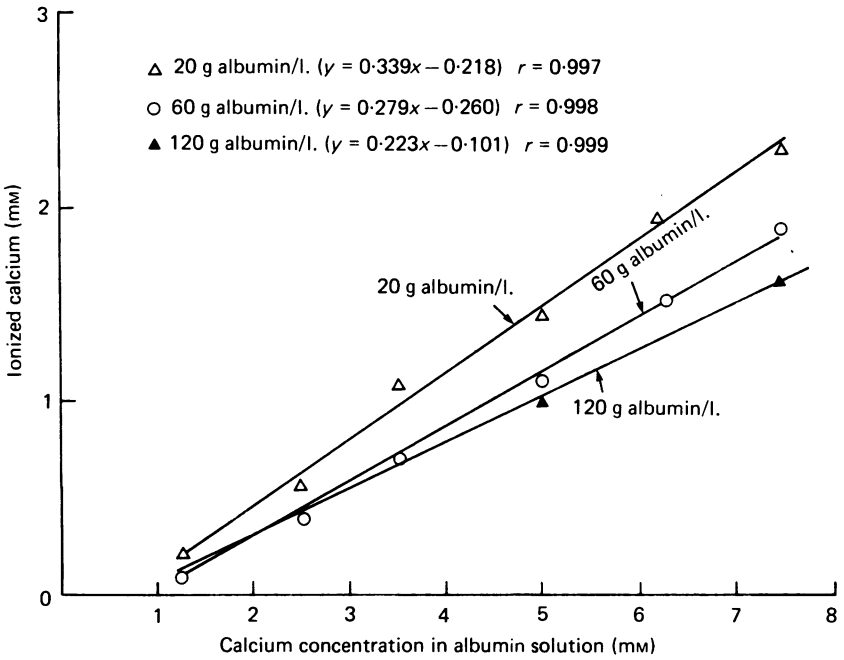

Fig. 2. Effect of raising calcium in perfusion medium on free ionized calcium. Perfusion medium contained either 20, 60 or 120 g albumin/l. Each point represents at least three separate determinations using a calcium-sensitive electrode. Standard deviations were too small to be plotted on the graph.

expected, and that calcium concentration in the 20 and 60 g albumin/l. medium must be raised to 6·25 and 7·45 mM to achieve 1·82 mM-ionized calcium. Fig. 3 illustrates that in the presence of at least 1·82 mM-ionized calcium autoregulation obtained in medium containing either 20 or 60 g albumin/l., though perfusate flows at all pressures were consistently higher in the medium containing 60 g/l. ($P < 0·05$).

In medium containing 60 g albumin/l., autoregulation was observed with 1·82 mM-ionized calcium, but not with 1·80 mM (Fig. 4). Conversely, raising ionized calcium to 1·83 mM induced autoregulation, but at a greatly reduced flow ($P < 0·05$).

We also sought to determine by what mechanism raising albumin concentration increased perfusate flow. Fig. 5 shows that maintaining ionized calcium concentration constant at 1·82 mM lowered perfusate flow at all albumin concentrations, but did not prevent the increased flow. The increased flow was reversible. However, removing the renal capsule completely obliterated the increased perfusate flow observed with raising albumin concentration (Fig. 6).

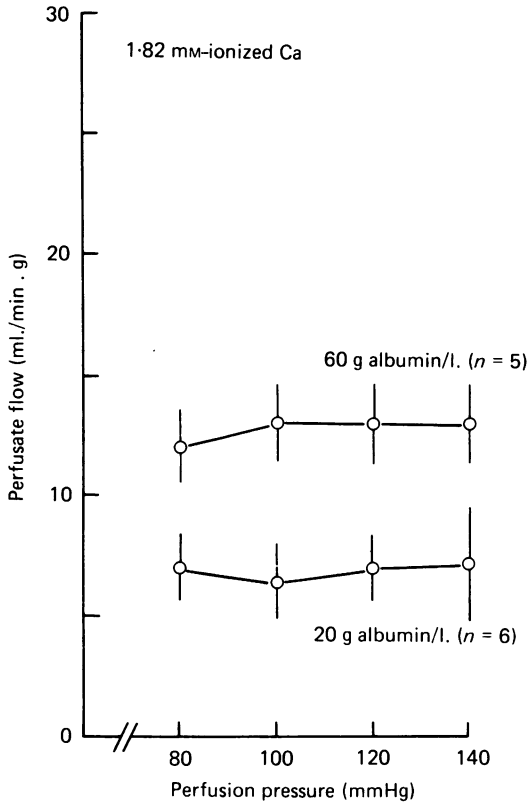

Fig. 3. Perfusate flow autoregulated in 20 or 60 g albumin/l. medium with 1·82 mM-ionized calcium. Each point represents mean ± S.E. of mean of the perfusate flow averaged over a 5 min perfusion period.

DISCUSSION

These studies provide clear evidence that autoregulation of renal perfusate flow is critically dependent on the ionized calcium concentration in the perfusion fluid. Below a certain level (usually 1·82 mM) autoregulation is not observed in medium containing albumin, but above this level it occurs though at a greatly reduced flow. Raising the albumin concentration lowers the ionized calcium below the level required for autoregulation, but replacing the calcium restores autoregulation to a certain extent. It is also clear that the mechanism of action of calcium involves transport of the ion, for the autoregulatory response can be blocked by the specific calcium transport blockers, verapamil and nifedipine (Ono *et al.* 1974). The renal afferent and efferent arterioles may be the major sites responsible for controlling renal blood flow in general and for autoregulation in particular (Navar, 1978; Wright & Briggs, 1979). Although these sites have also been implicated in controlling glomerular filtration rate, filtration is not required for blood flow autoregulation since blood flow autoregulation obtains in the complete absence of filtration (Sadowski & Wocial, 1977). It is for this reason and for others besides, that we have directed

our studies to elucidate the mechanisms controlling the autoregulation of renal perfusate flow without reporting or considering glomerular filtration rate.

We recognize that the perfusate flows reported here are somewhat higher than those observed *in vivo* (Navar, 1978); nevertheless, the fact that the flows are so strikingly dependent on extracellular calcium both *in vivo* (Chomdej *et al.* 1977) and *in vivo* (see

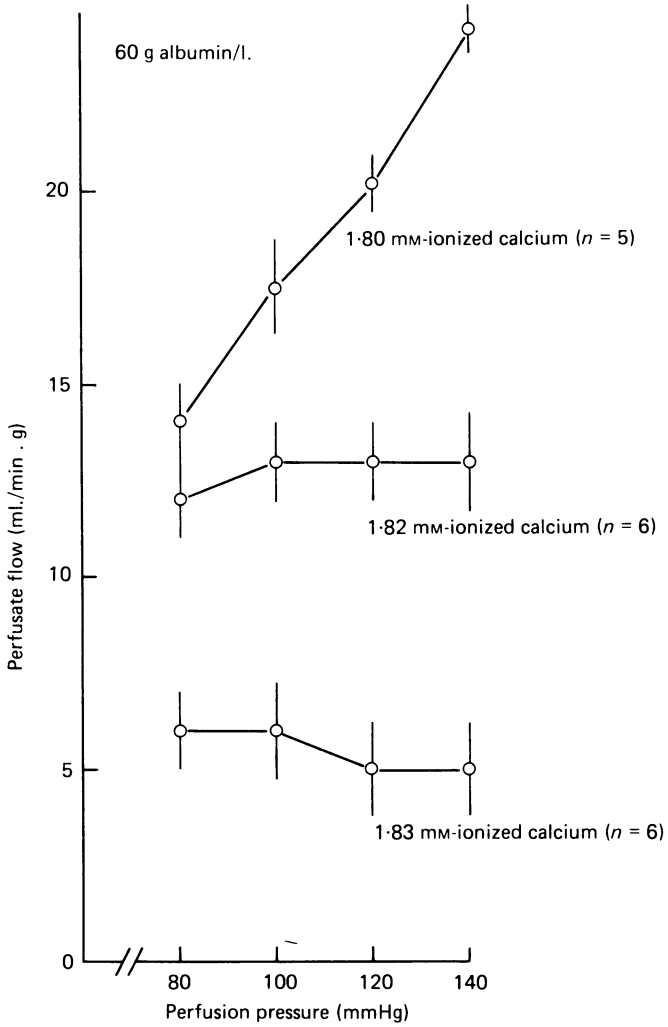

Fig. 4. Effect of raising ionized calcium concentration on autoregulation of renal perfusate flow in kidneys perfused with medium containing 60 g albumin/l. Each point represents mean ± S.E. of mean of the perfusate flow averaged over a 5 min perfusion period.

Fig. 4) suggests that one of the reasons for the high perfusate flow in isolated perfused kidneys with artificial perfusion fluid (Ross, Epstein & Leaf, 1973; Fray, 1976; DeMello & Maack, 1976; Ross, 1978; Fray & Karuza, 1980) might be related to the concentration of ionized calcium in the perfusion fluid, though the concentration of albumin or other oncotic agents does play a role (see Fig. 1). Recently, Maack (1980) has suggested that the low viscosity of the perfusion fluid accounts for the high

perfusate flow. Our observations do not support his hypothesis, for perfusate flow in the more viscous 120 g albumin/l. medium was much higher than the less viscous albumin-free medium.

One possible mechanism or site of action of calcium in controlling renal blood flow is the macula densa, as suggested by some workers (Israelit *et al.* 1973; Burke *et al.*

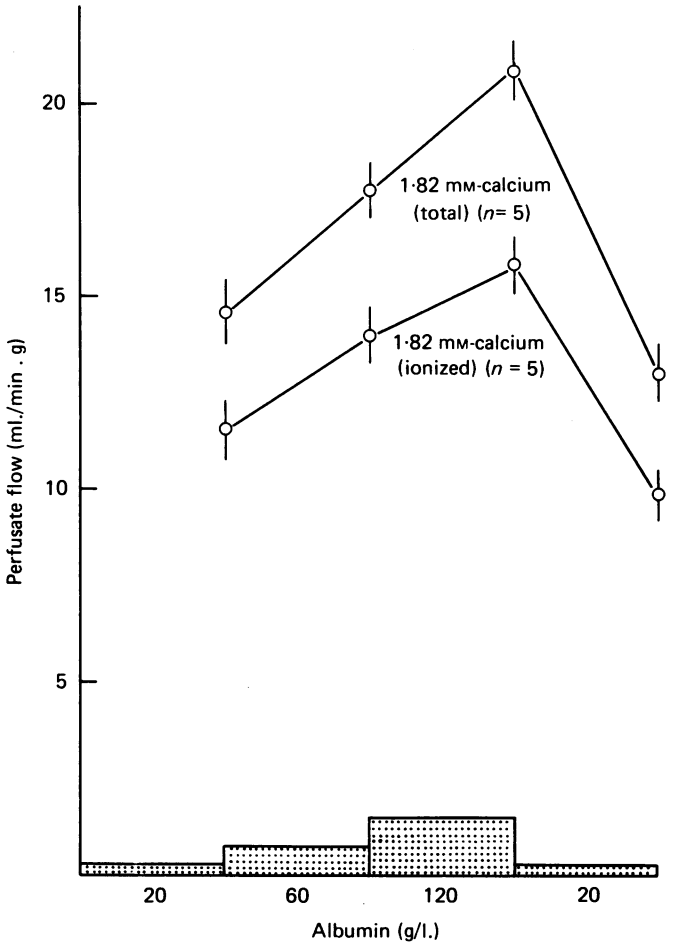

Fig. 5. Effect of raising albumin concentration on renal perfusate flow in the presence of 1·82 mM-total calcium and 1·82 mM-ionized calcium. Each point represents mean ± S.E. of mean of the flow averaged over a 5 min perfusion period. Perfusate flow at each albumin concentration with 1·82 mM-*ionized* calcium is significantly lower than that with 1·82 mM-*total* calcium ($P < 0.05$). Perfusion pressure was 100 mmHg.

1974). Though the macula densa has been postulated to control autoregulation by sampling some function of the distal tubular fluid, presumably sodium chloride or tubular flow rate (Navar, 1978; Wright & Briggs, 1979), calcium might also be a stimulus. This does not appear to be the case, however, since directly infusing calcium into the distal tubular fluid passing the macula densa does not induce the response (Schnermann & Hermle, 1975). Autoregulation of blood flow obtains even in the

complete absence of any fluid constituent at the macula densa (Sadowski & Wocial, 1977), providing additional evidence that the macula densa might not be the primary mechanism mediating the calcium response.

Considering the considerable amount of information concerning direct and specific effects of calcium on smooth muscle contraction, it is difficult to circumvent the

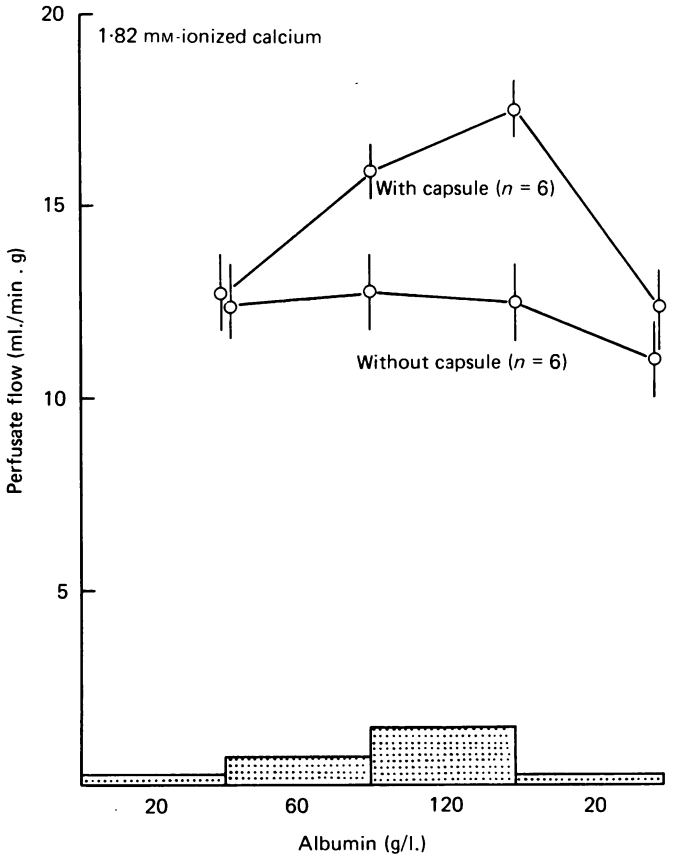

Fig. 6. Effect of removing the renal capsule on perfusate flow during high concentrations of albumin. Each point represents mean ± S.E. of mean of the flow averaged over a 5 min perfusion period. Perfusion pressure was 100 mmHg and ionized calcium was 1·82 mM. Perfusate flows during 60 and 120 g albumin/l. were significantly lower in kidneys without capsules ($P < 0·05$).

possibility that the first and foremost effect of calcium is directed at the smooth muscles of the afferent arteriole. Therefore, we propose that the autoregulatory component of the smooth muscles of the afferent arteriole in the isolated perfused rat kidney is exquisitely sensitive to changes in extracellular ionized calcium above a certain value. These experiments suggest that this value may be 1·82 mM-ionized calcium when kidneys are perfused with 60 g albumin/l. Below this value, autoregulation does not occur, but above this value, it occurs though at a greatly reduced flow.

Another aspect of these results that deserves emphasis is the mechanism by which hyperalbuminaemia abolishes blood flow autoregulation and increases renal blood

flow (see Fig. 1). One possible mechanism involves the calcium binding capacity of albumin (see Fig. 2). Thus raising albumin concentration lowers the concentration of ionized calcium in the extracellular fluid below the level required for autoregulation. Replacing the calcium restores autoregulation (see Fig. 3). In fact, replacing calcium beyond a certain limit still restores autoregulation, but at a greatly reduced flow. A similar phenomenon has been observed in dogs (Chomdej *et al.* 1977). Another possible mechanism involves the effect of albumin or other oncotic agents on intrarenal tissue pressure. Hyperoncotic dextran, and presumably albumin, raises intrarenal tissue pressure (Ott, Navar & Guyton, 1971). We might expect, then, that removing the renal capsule should abolish the increased flow induced by raising albumin concentration. This has been observed (see Fig. 6). Thus, hyperalbuminaemia raises renal blood flow and abolishes autoregulation by lowering the extracellular ionized calcium concentration and by raising the intrarenal tissue pressure.

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