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THE USE OF REGIONAL HEPARINIZATION FOR PERFUSION OF THE KIDNEY *IN SITU*

BY

F. CARSWELL, R. HAINSWORTH AND J. R. LEDSOME

From the Department of Physiology, School of Medicine, University of Leeds

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When an organ is perfused with blood passing through an extracorporeal circuit, use of an anticoagulant technique is necessary. Heparinization of the whole animal prevents coagulation of blood but may lead to continuous loss of blood from the operation sites.

A continuous local anticoagulant effect may be produced by the infusion of heparin into the perfusion circuit and the neutralization of the heparin by infusion of protamine into the rest of the body. Apart from the original description (Gordon, Richards & Perkins, 1956) of the use of this technique of regional heparinization in limb perfusion, few reports have appeared of the use of the technique in a functioning vascular circuit or organ. The present paper shows that this technique can be used in a study of the canine kidney perfused *in situ*. A short report has already been given (Carswell, Hainsworth & Ledsome, 1967).

METHODS

The experiments were carried out on 29 dogs weighing 12.7–36 kg. The dogs were given a subcutaneous injection of morphine sulphate (0.5 mg/kg) and 1 hr later under local anaesthesia (decicain 2%) a catheter was inserted through a saphenous vein into the inferior vena cava. Each animal was anaesthetized by an intravenous infusion of a solution of chloralose (British Drug Houses: dose 100 mg/kg body weight) 1 g/100 ml. in sodium chloride solution (0.6 g/100 ml.). Subsequently during the experimental procedures a steady state of light anaesthesia and fluid input was maintained by the infusion every 10 min of 1.5 ml./kg body weight of either sodium chloride solution (0.6 g/100 ml.) or chloralose solution. Some animals breathed spontaneously, in others the chest was opened and artificial respiration was started with a mixture of 40% oxygen in air, humidified at room temperature and supplied from a Starling Ideal pump the rate (18/min) and stroke (about 50 ml/3 kg body weight) of which were adjusted to equal approximately that of the intact animal's spontaneous respiration.

The right ureter was catheterized through a small incision in the right loin. The left kidney, which was to be perfused, was approached through the left loin. In the initial experiments the double lumen cannulating technique described by Langston, Guyton & Gillespie (1959) was used for perfusion. In the later experiments the left renal artery was tied at its origin from the aorta and cannulated distally with a stainless steel cannula. Blood was pumped from a stainless steel cannula in the left femoral artery by a variable rate roller pump (Watson-Marlow Ltd., M.H.R.E.) into a reservoir in which the air above the blood was maintained at constant pressure. The flow of blood into the pressure reservoir was controlled by altering the pump rate either manually or by means of an on–off switch actuated by the blood closing the gap between two contacts when a particular height was reached in the reservoir. Blood flowed from the constant pressure reservoir into the left renal artery. About 4 min elapsed between the tying of the renal artery and the

re-establishment of renal blood flow. The volume of blood in the extracorporeal circuit ranged from 27 to 85 ml. but was constant in any one experiment.

Heparin (heparin B.P. mucous) was infused into the extracorporeal circuit at the site of removal of blood from the dog. The dose of heparin used was from 0.1–0.5 mg/kg body weight/min (100 i.u. being taken as equivalent to 1 mg). Because the dose of heparin should be related to blood flow in the circuit (30–360 ml./min) rather than to body weight and because blood flow could not be predicted, for convenience all solutions were prepared as for 15 kg dogs. Thus in most experiments the dose of heparin was 3 mg/min regardless of body weight. The heparin was diluted in NaCl solution (0.9 g/100 ml.), so that the volume infused was 0.5 ml./min.

Protamine sulphate (protamine sulphate injection, B.P.) was infused either into the renal artery at the end of the extracorporeal circuit or was infused into the inferior vena cava at the level of the renal veins in a dose adequate to neutralize the heparin. A heparin to protamine ratio of 5:6 by weight was used in all experiments. Protamine was diluted in NaCl solution (0.9 g/100 ml.), so that the volume infused was 1 ml./min.

The infusions were given from constant volume infusion syringes (B. Braun, West Germany). The heparin infusion was started immediately before cannulation of the femoral artery and the protamine infusion was started as soon as flow began from the extracorporeal circuit to the kidney. The protamine infusion was run at twice the normal rate for a time equivalent to that during which only heparin was infused. Initially samples of blood were taken from the extracorporeal circuit and from the systemic circulation at intervals of 15–30 min. Subsequently samples were taken at longer intervals. Regional heparinization was held to have been satisfactorily achieved when the clotting time in the perfusion circuit was persistently three times longer than that in the systemic circulation. Clotting times were measured by a modification of the method of Lee & White (1913). A 1 ml. sample of blood removed with a clean dry syringe was placed in a clean dry tube which was stoppered. The tube was inverted every 2 min until 10 min and every 5 min thereafter until 60 min. Clotting was said to have occurred when the contents of the inverted tube did not fall down the tube. Specimens not clotting by 60 min were recorded as clotting at 60 min.

Pressure in the renal artery was measured through a stainless steel tube (0.5 mm bore) which led into the renal artery cannula and opened with a lateral hole close to (within 2 mm) of the tip of the arterial cannula. Systemic arterial pressure was recorded through either a metal (Inconel, Johnson Matthey & Co., 1.5 mm bore) or nylon (Portex No. 4, 6 in. long) cannula in the right femoral artery and pressure in the inferior vena cava was recorded through a nylon catheter placed so that its tip lay close to the renal veins. To each cannula was attached a Statham strain gauge (model P23Gb) and after amplification by means of a carrier amplifier (S.E. Laboratories, Feltham, Middlesex) the pressure was recorded on an ultra-violet light recorder (S.E. Laboratories). Mean pressures were obtained electrically by passing the output of the amplifiers through an R-C network. Blood flow in the perfusion circuit was measured using an electromagnetic flowmeter (Statham Inst. Inc., Medicon M-4000). A cannulating probe was incorporated in the extracorporeal circuit between the blood reservoir and the renal artery. Zero flow was checked at intervals during the experiment and the flow meter was calibrated at the end of the experiment using blood from the experimental animal.

Urine was collected from each ureteric cannula and the volumes measured at 10 min intervals. In experiments in which the urine flow was small, an intravenous infusion of mannitol (125 mg/min in 0.5 ml. water) was given and maintained throughout the experimental period. The sodium concentration in the urine was measured using a sodium electrode (Electronic Instruments Ltd., BH 104 glass) and Vibron electrometer (33 E.I.L.) and a pH measuring unit (C33B E.I.L.). The electrode was calibrated using gravimetrically prepared sodium chloride solutions covering the sodium concentration range 1–400 mM. The results obtained by this method for urinary sodium are slightly lower than those obtained by the flame photometer (Moore & Wilson, 1963) but the ranking order is unaffected. Thus changes in urinary sodium concentration are reliably indicated by the electrode.

At the end of the experiment the kidneys were removed, sliced and inspected for microscopic evidence of infarction. Both kidneys were weighed. The extracorporeal circuit was also examined for clots after the blood had been washed out; in some experiments a filter was incorporated in the circuit (Baxter Laboratories, pore size 200 μ).

RESULTS

Regional heparinization

Regional heparinization of an extracorporeal circuit used in kidney perfusion was satisfactorily achieved in twenty-six out of twenty-nine dogs. This regional heparinization was achieved with infusion of 3 mg/min of heparin at blood flows of up to 360 ml./min. The maximum blood flow recorded was 234 ml./min (average of thirteen dogs). The causes of failure of regional heparinization either throughout the experiment or for brief periods were chiefly technical difficulties in the delivery of the heparin and protamine rather than the failure of the biological activity. Thus failure was attributed to faulty dosage (two), syringe leakage (one), clot at protamine inlet (three). In addition in two dogs given an infusion of oxalated blood from a donor dog there was difficulty in maintaining regional heparinization. Leakage of the syringes at high pressure occurred in an early experiment and was reduced by greasing the syringes with silicone high vacuum grease (Edwards Ltd.). A typical satisfactory result is shown in Fig. 1.

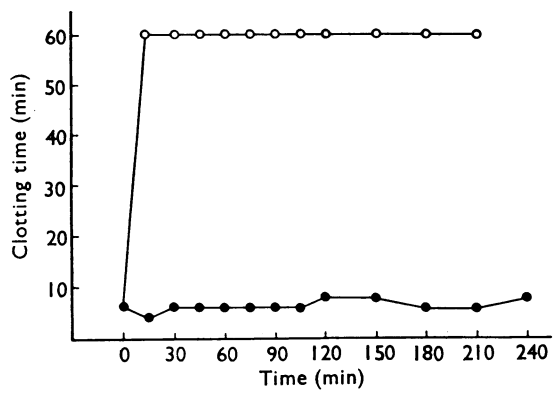

Fig. 1. Measurements of clotting time in blood from the systemic circulation (●) and blood from the renal perfusion circuit (○). Perfusion of the kidney started at zero time.

Examination of the extracorporeal circuit revealed soft clots on the filter on six occasions. Prior siliconization (Silicone M.S. Antifoam A., Hopkins & Williams) of the filter made these deposits unusual, however, and it seemed likely that the filter was promoting their formation; the filter was removed for the later experiments. On one occasion a clot appeared in the circuit when perfusion was started, having apparently formed in the femoral arterial cannula; on this occasion the heparin infusion had not been started as soon as the femoral artery was cannulated. The constant pressure device used necessitated the application of a small voltage (up to 50 mV) across two terminals in the heparinized blood in the reservoir; this produced a deposit on these terminals.

Examination of the perfused kidneys *post mortem* showed possible embolization on only two occasions in the twenty-nine experiments. On one of these occasions the switch on the heparin syringe had failed and a clot had formed at the heparin inlet.

The average duration of satisfactory regional heparinization of the renal perfusion circuit was 193 min and the longest time 470 min. No differences in ease of control were detected in those preparations in which protamine was infused into the renal artery as compared with those in which it was infused intravenously.

Vascular effects of protamine and heparin. To study the effects of protamine and heparin on renal vascular resistance, the constant pressure reservoir was isolated from the perfusion circuit and the kidney was perfused at constant flow by the roller pump. Thus any change in resistance to blood flow was indicated by a change in perfusion pressure.

Infusion of additional protamine (3–11 mg/min) into the renal artery produced a marked rise in renal arterial pressure (nine tests in three dogs). The record of one such experiment is shown in Fig. 2. The increase in renal vascular resistance was only partially reversible when the additional protamine was stopped. This increase in the dose of protamine infused only increased the total protamine dose by 2–3 times. No changes in heart rate or systemic arterial pressure were seen.

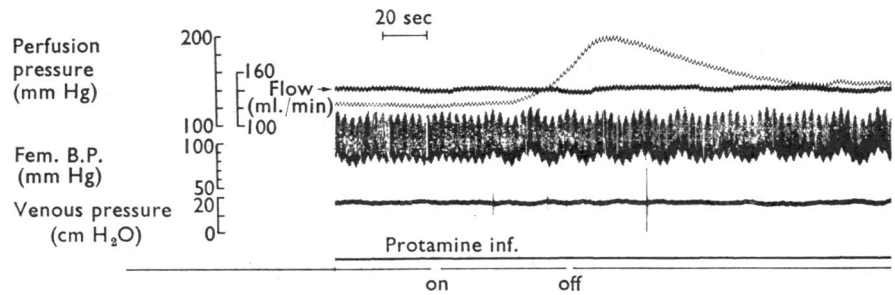

Fig. 2. Effects of an infusion of protamine (9 mg/min) on renal arterial perfusion pressure at constant blood flow.

In three dogs in which only heparin was being infused into the perfusion circuit, infusing protamine into the renal artery in a dose just adequate to neutralize the heparin caused a rise in renal perfusion pressure at constant blood flow in four out of five tests.

Intravenous infusion of protamine at twice the normal infusion rate, at the start of perfusion, did not produce any change in blood pressure, heart rate or renal perfusion pressure at constant blood flow (five out of six dogs). Large doses of protamine (20–100 mg) infused intravenously in 1 min produced a fall in systemic arterial pressure and an increase in renal perfusion pressure in six dogs in which this was tested. One dog in which 100 mg was given developed severe hypotension and died. Similar effects were described by Thompson (1900).

The rate of infusion of heparin into the perfusion circuit was increased in nine dogs by doses ranging from 4 to 40 mg/min. In eleven tests in the nine dogs there was no consistent change ; in five tests renal perfusion pressure did not change, in two tests there was a fall and in four tests an increase in perfusion pressure.

To test for possible effects of the heparin-protamine mixture, a previously mixed solution of heparin and protamine was infused into the renal artery at twice the normal rate. No changes in renal perfusion pressure were detected. Mixing heparin and protamine *in vitro,* however, produces a dense white gelatinous precipitate which adheres strongly to the walls of containers, so the actual dose reaching the animal is unknown.

During the course of a long perfusion there is probably an increasing *in vivo* concentration of the heparin and protamine mixture, but the gradual increase in renal vascular resistance occurring during the course of the perfusion (see below) did not seem to be any greater than that which has been reported by other workers using general heparinization (for example, Selkurt, 1951). No differences in responses to occluding the carotid arteries and increasing the renal perfusion pressure were observed between kidneys in which regional heparinization was produced by intra-arterial infusion as compared with intravenous infusion.

Functional assessment of the kidney

Renal blood flow. In eight dogs measurements were made of renal blood flow at constant perfusion pressure over the first 3 hr of perfusion. The average blood flow at the start of perfusion was 224 ml./min, and this had fallen to 181 ml./min after perfusion for 3 hr. The average weight of the perfused kidneys in these experiments was 63.5 g (range 47.3–86.0).

Renal nerves. Care was taken during dissection around the renal artery not to damage the renal nerves. In eight dogs in which occlusion of both carotid arteries produced a rise in systemic arterial pressure there was a rise in renal perfusion pressure at constant flow. An example of this response is shown in Fig. 3. The rapid time course of the change

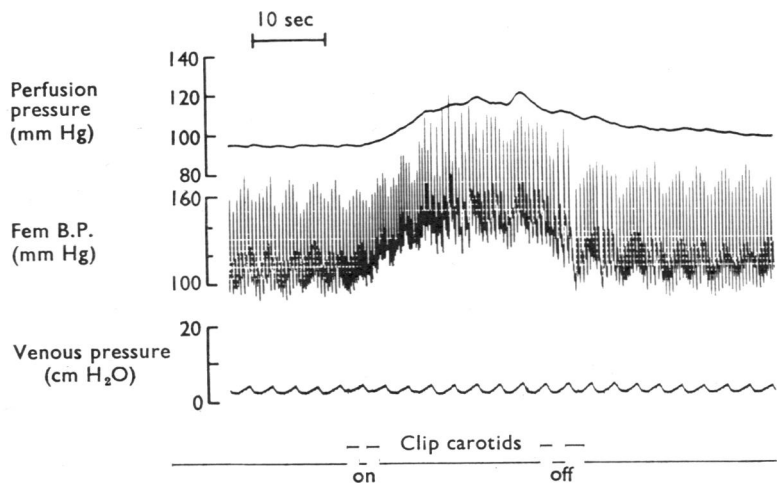

Fig. 3. Effects of occlusion of both carotid arteries on renal perfusion pressure, femoral arterial pressure and venous pressure at constant renal blood flow. Regional heparinization with intravenous protamine infusion.

in renal arterial pressure is such that only a change in activity in renal nerves could have produced the response. The perfusion circuit introduced a delay in the transfer of blood from the systemic circulation to the kidney of 10–30 sec. This response could still be demonstrated after 8 hr of regional heparinization.

Urinary sodium excretion. The urinary sodium concentration ranged from 1.8 to 235.0 mM in these dogs. The increase in urinary sodium excretion which occurs when

renal arterial perfusion pressure is raised has been well described (Selkurt, 1951). In ten tests in seven dogs the renal arterial perfusion pressure was raised for 10–40 min. The average " low " perfusion pressure was 114 mm Hg (range 85–154) and the average " high " perfusion pressure was 172 mm Hg (range 139–200). The results of one experiment are shown in Fig. 4. The mean urine volume before raising the perfusion pressure was 0.6 ml./min (range 0.07–1.50); on raising the perfusion pressure the mean urine volume was 0.99 ml./min (range 0.1–2.20); on lowering the perfusion pressure again the urine volume was 0.73 ml./min (range 0.06–1.63). The urine sodium concentration was 13.4 mM (range 1.90–39.0) before raising the pressure; on raising the pressure it was 28.4 mM (range 7–70); on lowering the pressure it was 20.6 mM (range 1.9–32.0). An increase in sodium excretion was produced on every occasion by raising the perfusion pressure.

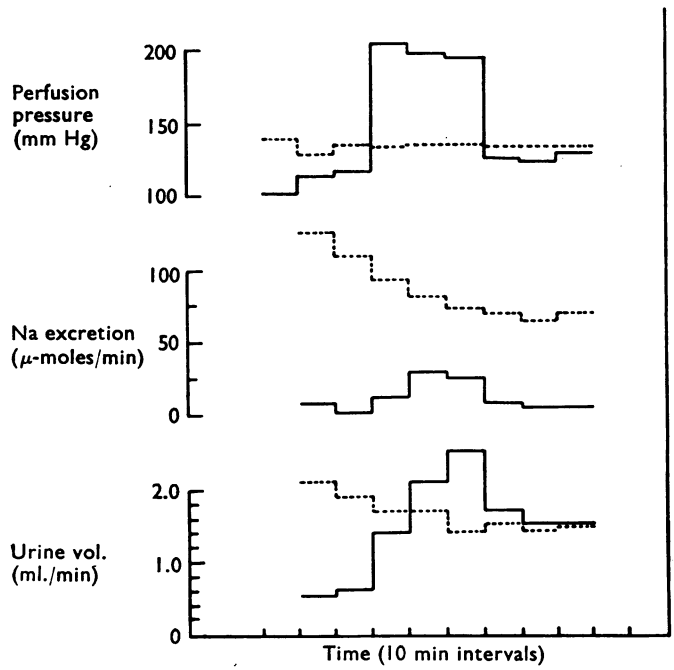

Fig. 4. Effects of increasing renal arterial perfusion pressure on sodium excretion and urine volume. Values for left kidney ——— (perfused and regional heparinization); values for right kidney - - - - - (control).

In Fig. 4, the sodium excretion of the two kidneys has been compared at similar perfusion pressures, the right kidney being perfused by the systemic pressure and the left kidney being perfused from the extracorporeal circuit at a similar mean pressure. In seven out of eleven dogs the average sodium excretion was lower in the perfused than in the normal kidney; in the other four dogs the average sodium excretion was similar in the two kidneys.

DISCUSSION

The term regional heparinization was used by Murray & Best (1938) to describe the local arterial injection of small doses of heparin, sufficient to affect the clotting time locally, but insufficient to significantly change the clotting time of the whole blood stream.

More recently Gordon, Richards & Perkins (1956) used the term to describe a system in which the clotting time could be prolonged by local arterial injection of heparin which was neutralized by protamine in the systemic circulation.

Experiments involving measurements of renal excretion require long experimental periods for the collection of adequate control and experimental urine specimens. One cause of the deterioration of such preparations could be continuous loss of blood from surgical wounds; if regional heparinization reduces the loss of blood then it may be possible to reduce the rate of deterioration of the preparation. Before the technique can be used in experiments on renal function, however, it must be shown that the heparin and protamine do not have undesirable effects on the kidney and that the kidneys respond to physiological stimuli in a similar way to kidneys perfused in animals with general heparinization.

The experiments described show that heparinization of the perfused kidney can be achieved while the clotting time in the blood of the systemic circulation is within normal limits. Early difficulties were chiefly the result of technical failures resulting in the administration of incorrect doses of heparin and protamine. Measurements of clotting times made at intervals throughout the experiment were adequate to allow adjustments to be made to the rate of infusion of heparin and protamine as necessary. The major aim of heparinization in these experiments was to prevent clot formation in the perfusion circuit, and the dose of heparin used was of the order of 1–2 mg/100 ml. blood flow. This dose prolonged clotting time beyond 60 min in most experiments; in only two experiments was there any macroscopic evidence of infarction in the kidney; in one of these the heparin infusion had failed. In their original description of the method, Gordon *et al.* (1956) used doses of heparin corresponding to an estimated 3 mg/100 ml. blood flow in perfusion of a dog hind limb. In a subsequent paper, Gordon, Simon, Rukes, Richards & Perkins (1956) used 0.5 mg/100 ml. blood flow to maintain regional heparinization in an artificial kidney circuit. Smaller doses than these have been reported more recently. Thus, Maker, Lapierre, Schreiner, Geiger & Westerveldt (1963) used doses of 0.2 mg/100 ml. blood flow in human subjects undergoing haemodialysis but reported clot formation in 31.7% of cases. It is not clear whether these were true clots or merely fibrin deposits. The technique has also been used in perfusion of animal liver (Eisenman, Liem & Raffucci, 1965); these workers used 0.1 mg/100 ml. blood flow and reported satisfactory results. The major aim of these latter two groups of workers has been to avoid elevation of clotting time in the systemic circulation because the subjects were patients in whom the risk of bleeding was high and normal clotting mechanisms were not present.

Initially it was thought desirable to have a normal clotting time in the blood perfusing the kidney and for this reason protamine was infused into the renal artery cannula rather than being given intravenously. The experiments showed that such an infusion of protamine caused an increase in renal vascular resistance even in doses only just adequate to neutralize the heparin. Doubling the dose of protamine caused a marked increase in renal vascular resistance. The mechanism involved in the increase in renal vascular resistance is unknown. Shelley, Hodgkinson & Visscher (1942) reported the formation of a white precipitate when protamine was added to plasma and also reported agglutination of corpuscles in whole blood. The addition of protamine to dog blood perfusing the guinea-pig liver and lung caused a total cessation of blood flow. Very large amounts of

protamine were added, however: 10–60 mg protamine into a blood flow of 10–20 ml./min or about 100 mg/100 ml. blood flow. The authors attributed the cessation of blood flow to the vascular occlusive effects of the precipitate and the agglutination of the corpuscles. The amounts used in the present experiments were much less, 1–2 mg/100 ml. blood flow. Thus the increase in renal vascular resistance may have been caused by vascular occlusion but may represent an effect of protamine on the renal vessel walls. The consequent increase in renal vascular resistance makes the infusion of protamine into the renal perfusion circuit undesirable. No changes in renal vascular resistance could be demonstrated when protamine was given intravenously unless large doses were used; these caused effects upon the systemic circulation. Thus it is recommended that protamine should be infused intravenously to neutralize the heparin.

The reflex vascular responses of the kidney to occlusion of the carotid arteries have been fully described previously (Page & McCubbin, 1953). The kidneys perfused using regional heparinization had an active nerve supply as indicated by increased renal vascular resistance during carotid occlusion. This response could still be demonstrated after 8 hr of perfusion. There was also an increase in sodium excretion by the kidney when the perfusion pressure was raised; this response of the perfused kidney has been described in dogs with general heparinization (Selkurt, 1951). It is concluded that kidneys perfused using the technique of regional heparinization will respond to these physiological stimuli in a manner similar to kidneys perfused in animals with general heparinization.

SUMMARY

1. The technique of regional heparinization has been used for the perfusion of the kidney *in situ*.

2. Infusion of protamine into the renal perfusion circuit caused an increase in renal vascular resistance. It is recommended that for neutralization of heparin in the systemic blood protamine be infused intravenously.

3. Kidneys perfused using this technique had blood flows within the range 30–360 ml./min and showed normal responses to carotid occlusion and to an increase in renal perfusion pressure.

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