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Interim Report on a Method of Extracorporeal Perfusion of Preserved Canine Kidney

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Summary. The early renal function after preservation with cold C3 Collins solution has been studied by means of kidney perfusion on a high-flow external arteriovenous shunt. With this technique, the authors have been able to establish that a measurable GFR is almost immediatly reached after connection even if some tubular impairment is present, and if the O_2 consumption reduced. At microscopy, normal glomeruli and peculiar tubular vacuolization have been seen, probably an expression of ischaemic damage. The experimental model has proved very simple and suitable for many other studies.

Key words: Renal preservation, hypothermic storage of organs, extra corporeal perfusion.

Autotransplantation for assessing the viability of preserved organs is included in most experimental models upon which the studies in renal preservation have been based. Unfortunatly, many uncontrollable factors in experimental kidney transplantation lead to great variability in results.

To partly overcome these difficulties, some authorities have successfully carried out the perfusion of the kidney on an arteriovenous shunt after preservation (4, 10).

The present experience was undertaken to study the early renal function after a period of preservation with Collins C3 solution (6), by means of cannulation of the femoral artery and vein.

Materials and Methods

Ten male and female mongrel dogs, weighing 12 to 27 kg, were anaesthetized and the kidneys were approached through a midline transperitoneal incision. Both ureters were divided and catheterized and separate renal function was determined.

Unilateral nephrectomy of the kidney provided with a single artery was then performed after decapsulation, and the renal artery immediatly cannulated. The kidney was flushed from a height of 100 cm with cold C3 solution (4° C). Finally a ureterocutaneostomy of the control kidney left in situ and an arteriovenous external shunt between femoral vessels were accomplished.

During the storage time the dogs were maintained under light anaesthesia and positive pressure with high percentage of oxygen: the blood pressure, acid-base balance and renal function being continuously monitored.

The preserved kidney was afterwards connected to the cannulae of the A-V shunt and the bilateral renal function tested for a period of about an hour by the following methods.

At 20 min intervals, endogenous creatinine clearances, urea clearances, plasma and urine osmolalities, electrolyte excretion and the A-V differences in oxygen content of the cannulae were measured (°).

After the first period of extra-corporeal perfusion of the preserved kidney, a hypertonic solution of 5% sodium chloride was infused at the rate of 1 ml/min to enhance diuresis.

The systemic arterial pressure and the pressure of the arterial cannula were continuously recorded whereas arterial and venous flows were estimated at the start and at the end of the experiments by

(°) Creatinine was estimated using the Technicon autoanalyzer method, osmolalities by means of a Fiske osmometer, electrolytes with a EEL flame photometer and a EEL choride meter, oxygen and acid-base balances with a Corning EEL gas meter.

direct collection (Figs. 1 and 2). Both kidney were ultimately fixed by arterial infusion of formalin for microscopic examination.

Results

In nine of ten preserved kidneys a good blood flow, averaging 3 ml/mg of tissue, was soon established after the release of the arterial and venous clamps (Fig. 2).

From these kidneys a detectable diuresis occured within 1-3 min of the start of extracorporeal perfusion. In the tenth case the urine output was always very poor: high intraparenchymal resistance with some imperfect blood drainage into the venous cannula characterized the perfusion.

The systemic arterial pressure was in any case in the normal range not necessitating any administration of vasoactive or nephrotoxic drugs.

The arterial and venous oxygen contents of the cannulae, evaluated by simultaneous sampling (Fig. 1), were found to be very high, depending upon the O_2 ventilation, as well as the A-V differences in pO_2 (mean arterial pO_2 = 350 mmHg; mean venous pO_2 = 110 mmHg).

Fig. 3 shows the creatinine clearances of preserved kidneys compared with the prestorage values. In Fig. 4, mean creatinine clearances of control kidneys are registered with the contemporary values of the preserved organs.

The figures concerning solute excretion showed that for the kidney left in situ at the end of the six-hours period of preservation, in the fully hydrated animal, the ratio U/P osm. was 2.0 or more with a mean urine osmolality of 450 mOsm./kg, whereas, for the preserved kidney, these ratios were less than 1.2 in all the cases, with a mean urinary osmolality of 280 mOsm./kg. The above mentioned values did not vary during the extracorporeal shunt perfusion.

The pH determinations on the arterial and venous cannulae showed a clear diminution of the values on the venous side with a parallel decrease in the base excess which was grossly negative in blood returning from the perfusing preserved kidney.

At optical microscopy, some enlargement of the Bowman space was noted the glomerular tufts were otherwise normal. In the same subject in which the venous drainage had been imperfect, the kidney was considerably congested. Only in this dog, compacted red blood cells were found in glomerular capillaries and in the peritubular interstitium, especially around distal tubules.

In kidneys preserved with Collins C3 solution, inconstant tubular changes were present, consisting of a quite unusual vacuolization of the cytoplasm without evidence of cellular swelling or lesions of nuclear bodies. No cellular sloughing into the lumen or other sign of tubular necrosis was observed.

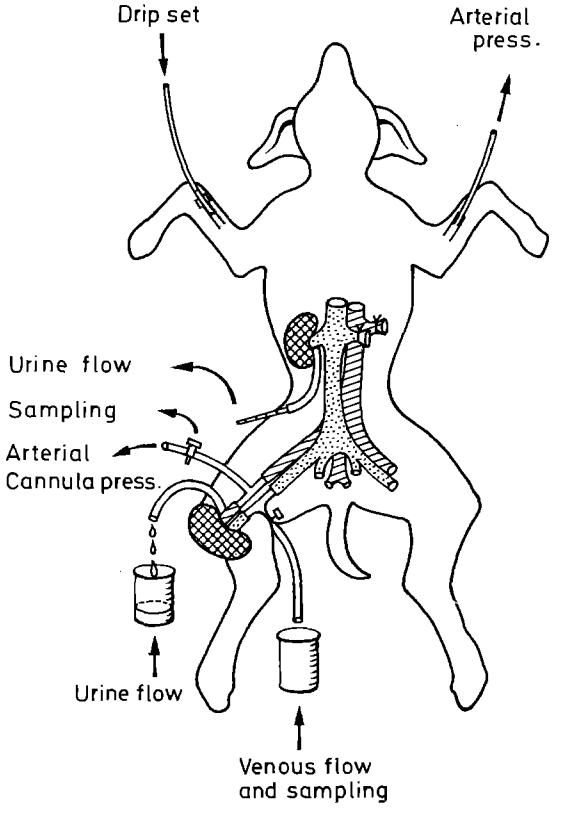

Fig. 1. Experimental arrangement

Discussion

It has been shown in present and other studies that normothermic perfusion of preserved canine kidneys on a high-flow arteriovenous shunt is a simple experimental model (4).

Obviously, the observations made available by this short-term perfusion are quite different from those collected after reimplantation, the latter method giving complete information on the longterm viability of the preserved organs.

With this technique we have been able to confirm the efficacy of the C3 Collins solution as a preservation medium at low temperature, making at the same time some interesting observations on the distinctive features of the early functional recovery of the preserved kidney.

So, in Fig. 3, one can see that the kidneys gained a measurable glomerular filtration very soon after the vascular connection. The mean GFR reached in an hour was about the 50 % of values registered before the preservation. The figures of GFR compared with those of the controlateral kidney left in situ were only slightly lower (Fig. 4). By means of the study of solute excretion, a predictable impairement of concentrating ability for all preserved organs has been found. The hypotonicity

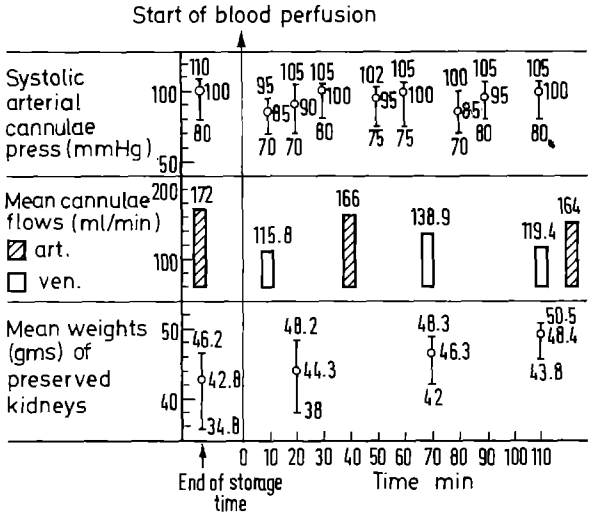

Fig. 2. Data concerning the blood arterial pressure, arterial and venous flows of the cannulae. Some figures of the preserved organs weights are also reported

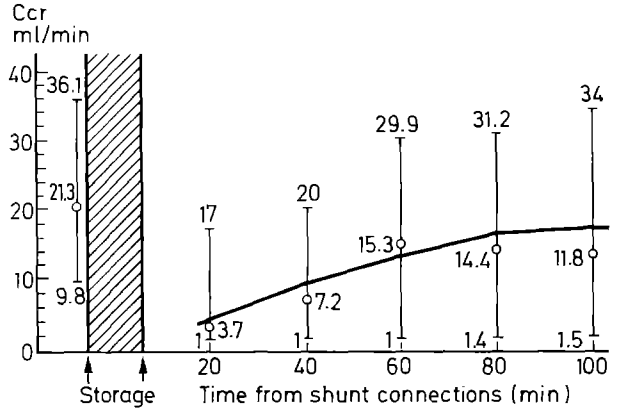

Fig. 3. Values of creatinine clearances of preserved kidneys

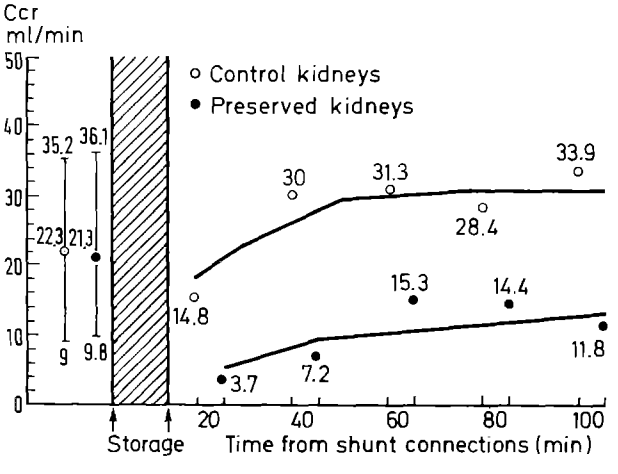

Fig. 4. Mean creatinine clearances of preserved and control kidneys

of urine did not vary during the observation time, which has been too short to exclude the possibility of any recovery and nor did the urinary excretion per minute any.

These findings have been easily explaned as a consequence of tubular damage, which was confirmed at histology and seemed to be the prominent lesion.

The behaviour of the arterial and venous pH and of base excess was accounted for by the obvious metabolic acidosis of the anoxyc parenchyma, even if oxygen exposure could in theory cause excessive release of hydrogen ions from perfusing dog kidney (8).

No clear interpretation of the arterial and venous figures of pO_2 has been possible because the prolonged, high-rate oxygen ventilation. The data is, any way, equal to that drawn from the isolated dog kidney perfused by various perfusate saturated with 100% of O_2 (8). By means of calculation of the quantity of O_2 dissolved in plasma at the very high arterial pO_2 registered, and utilizing the formula for indirect measure of oxygen consumption employing the polarographic determinations of O_2 on gas meters (°), we found that about 20 micro-liters of O_2 were consumed per gram of renal tissue per minute.

One cannot state in such unphysiological respiratory conditions, if these kind of values are really representative of the true respiratory coefficient of the preserved tissue, because so many uncontrolled variables interfere with the data collection (surface diffusion, urinary loss of oxygen due to the very high percentage of plasma-dissolved O_2, etc.). Nevertheless it is perhaps possible to deduce that the oxygen consumption of these organs is in some way decreased, depending on a persistence of tissue hypoxic conditions.

It is moreover clear that only experiments especially designed for this purpose (using oxygen electrode assembly, etc.) could fully investigate these particular problems (3, 12).

From the morphological point of view we also are uncertain about the origin of the tubular vacuolization mentioned. The lesions, almost confined in proximal tubules, like most early lesion in preserved kidneys (16), were probably in relation to the

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(°) $\text{diff } O_2 \text{ A-V} \times 3 \times \text{AF}$ (ml/g/min)

$$\frac{p_b - \text{W. V. p.}}{}$$

where: $\text{diff } O_2$ A-V = arterial pO_2-venous pO_2

AF = arterial renal flow

p_b = barometric pressure

W. V. p. = temperature corrected water vapor pressure.

ischaemic damage (1, 14) and were similar to those produced in so-called "osmotic nephrosis" (2, 7, 15): the perfusion at nephrectomy being indeed made with a hyper-osmotic fluid (mOsm./kg = 305 - 340).

Most of the data we have collected with the present experience deserves further investigation, but we are of the opinion that arteriovenous extracorporeal perfusion is a suitable technique not only for studying the remaining function in a preserved kidney and its immediate viability (5, 9), but also for testing in reproducible experiments various preserving media, minimizing the undoubted technical errors (13).

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