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Hypothermic Perfusion of Human Kidney Carcinoma: Effects on Viability and Perfusate Distribution*

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Abstract—*A more deteriorating effect on tumour tissue than on normal renal parenchyma by hypothermic perfusion has previously been observed. The purpose of this study was to further evaluate changes in the perfusate distribution and the viability of normal cortical tissue and of kidney carcinoma during hypothermic perfusion. Tumour-involved kidneys obtained by nephrectomy from 12 patients were studied either with regard to viability, as measured with the Na^+-K^+ pump function in incubated tissue slices, or with regard to perfusate flow, as analysed with an isotope labelled microsphere technique. Six* days *of hypothermic perfusion decreased the potassium content in the tumour tissue, while it was mainly unchanged in the cortical tissue. The steady-state level of potassium in incubated slices reflected that the transmembrane K^+ influx was unchanged after* 6 days *of perfusion in normal cortical tissue, whereas a significant reduction was observed in the tumour tissue. The relative perfusate flow in the tumour, when compared to the normal kidney cortex flow, was reduced from* 60% *after* 1 hr *of perfusion to* 30% *after* 6 days *of perfusion.*

INTRODUCTION

THE METABOLISM in human and dog kidneys during continuous hypothermic perfusion has previously been studied in order to improve the preservation of kidneys for transplantation [1, 2]. The human kidneys included in these studies were mostly tumour-involved kidneys. A spin-off product of these investigations was that differences were found in the utilization of glucose, amino acids and fatty acids between normal and neoplastic tissue. These results suggested that hypothermic perfusion caused a more profound decrease in protein synthesis in the tumour than in the normal parenchyma [3].

Whether these differences between normal renal parenchyma and tumour tissue reflect a different primary cellular response to the hypothermic perfusion or merely a change of perfusion distribution with secondary metabolic effects is not known.

The aim of the present study was to examine the distribution of the perfusate and the viability of normal cortical tissue and kidney carcinoma during prolonged hypothermic perfusion.

MATERIALS AND METHODS

Materials

Tumour-involved kidneys were obtained from 12 patients, 4 female and 8 male, with a mean age of 62 yr (range 37–75 yr). The preoperative examination included intravenous pyelography, renal angiography and cavography. Only angiographically well-vascularized tumour-kidneys were chosen but partial heterogenicity in the vascularization could not be excluded by this method. In no case was a tumour thrombus found in the renal vein.

Transperitoneal perifascial nephrectomy according to Wahlqvist was performed in all patients [4]. In order to reduce the ischemic

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period, the method was modified and the kidney was gently mobilized before clamping of the vessels. After division of the renal vessels the renal artery was immediately cannulated and the kidney was flushed with a mixture of 18 ml of 1% lidocainehydrochloride (Xylocain®, Astra, Sweden) and 2 ml heparin (5000 IU/ml) at room temperature followed by 5% low-molecular-weight dextran (Perfudex®, Pharmacia, Sweden) at +6°C until the venous effluent was clear. The kidney was then flushed with a mixture of 10% invert sugar solution (Invertos®, ACO, Sweden) and isotonic sodium bicarbonate ($NaHCO_3$, 14 mg/ml, v/v = 1/1) at +6°C. The kidney was rapidly transported to the laboratory and weighed (mean weight 295 g, range 164–540 g). A small biopsy was taken from the tumour for histopathological verification of the cancer diagnosis. In experiments on perfusate distribution no biopsies were taken until the perfusion study was finished and the kidney had been fixed (see below).

Perfusion procedure

The kidney was perfused at 8–10°C in a Gambro perfusion machine (Gambro PF 2 D, Lund, Sweden) in accordance with a previous description [2]. The perfusate was based on human albumin with the addition of electrolytes of extracellular concentrations, amino acids, fatty acids and glucose [5]. The 'systolic' pressure was kept at 60 mm Hg by adjusting the volume flow of the perfusion roller pump.

Tissue preparation and incubation procedures

Biopsies were taken from normal kidney cortex and the tumour after 1, 72 and 144 hr of hypothermic perfusion. The cortex was separated from the medulla by sharp dissection. Some cortical biopsies were used for determination of sodium, potassium and water content in unincubated tissue. From other biopsies slices with a thickness of 0.4 mm were prepared with a McIlwain tissue chopper (Mickle Laboratory Engineering Company, U.K.) for studying the content of ions and water in incubated tissue. The slices were rapidly rinsed in ice-chilled normal Krebs' solution and then were transferred to 25 ml Erlenmeyer flasks containing 10 ml normal Krebs' solution (mmol/l: NaCl 122, KCl 4.73, $CaCl_2$ 2.49, $MgCl_2$ 1.19, $NaHCO_3$ 15.5, KH_2PO_4 1.19 and Glucose 11.5) for incubation. Incubations were performed at 37°C in a metabolic shaker under constant agitation (20 count/min). During the incubation the flasks were continuously aerated with a gas mixture of 96% O_2 and 4% CO_2. After various times of incubation the content of potassium, sodium and water was determined.

The variegation of renal cell carcinoma with hemorrhagic, necrotic, calcified, fibrotic and cystic parts makes the choice of tissue difficult. The ambition was to choose viable tissue from different parts of the tumour. Consequently, 5 slices were always taken for the incubations.

The wet weight of the specimens was determined after blotting on a filter paper. Thereafter the specimens were dried to constant weight at 40°C (dry weight) and digested in concentrated HNO_3 for potassium determination in an Eppendorf flame photometer as described previously [6].

Perfusate flow analysis

Perfusate flow was studied using isotope-labelled microspheres [7] with a diameter of 15 μm. One hour after the start of perfusion, when perfusate flow and pressure were stable, approximately 5×10^5 spheres labelled with ^{103}Ru (~10 μCi, 497 keV gamma emission) were syringe-injected by puncture of the afferent rubber tubing 5–10 cm from the renal artery. No reference perfusate was drawn distal to the injection site and thus no absolute flow data could be obtained. Six days later another injection was performed using the same number of spheres, but this time labelled with ^{125}I (~10 μCi, 29–31 keV gamma emission).

Micronized barium sulphate (Barosperse®, Mallinckrodt INC, St. Louis, MT, U.S.A.) dispersed in saline was then perfused through the kidney at the same pressure as above, followed by perfusion with the same contrast medium now suspended in 4% formaldehyde solution.

The whole kidney, after removal of pericapsular tissue, was subjected to radiography (40 kV, ~150 mAs Industrex C, Kodak). Several 2- to 3-mm median sections then were cut through the kidney, including the tumour. These sections were also radiographed (27 kV, ~15 mAs Industrex C, Kodak) and one was used for autoradiography. From one direction the film (Industrex C, Kodak) was exposed to the section without filters while the film on the other side of the section was shielded by a lead filter 0.1 mm thick. Thus the former film was exposed to the gamma emission from both types of spheres while the latter was exposed only by those spheres injected at the start of the perfusion. If the relative density of cortical and tumour areas differs between the two autoradiograms it is an indication that the relative blood flow of these tissues has changed. For convenient visualization one of the films was reversed (negative to positive) and the contrast between unexposed film and cortex was adjusted to be similar for both films. Subtraction was made by superimposing the films ('supersubtraction').

Contrasts were now easy to observe and with this procedure an increased density related to the cortex indicates a decreased relative flow at the end of the perfusion. From an adjacent section several pieces from normal renal cortex and carcinoma were cut and radioactivity was measured in a well-type scintillation counter for determination of the relative distribution of perfusate. The tissue pieces were then processed for histopathology.

The perfusion set, which encountered several possible traps for microspheres, was measured in a Packard Armac scintillation counter.

Statistical methods

Conventional statistical methods were used to calculate the means and standard errors of the mean. The significance of difference between matched pairs was tested by the Wilcoxon signed rank test [8].

RESULTS

Tissue viability

The potassium content in cortical tissue and tumour tissue after hypothermic perfusion for 1, 72 and 144 hr is given in Fig. 1. In the normal tissue the potassium content was constant during 6 days of perfusion while a reduction occurred in the tumour tissue. Furthermore, the potassium content was significantly lower in the tumour tissue compared to the cortical tissue after 72 ($P<0.02$) and 44 ($P<0.01$) hr of perfusion, indicating a reduced capacity to maintain normal transmembrane potassium gradients in tumour tissue during perfusion.

As demonstrated in previous studies, the capacity for transmembrane K^+ influx in cortical tissue during incubation *in vitro* is reflected in the potassium content during steady-state conditions [9, 10]. Therefore changes in the capacity of the

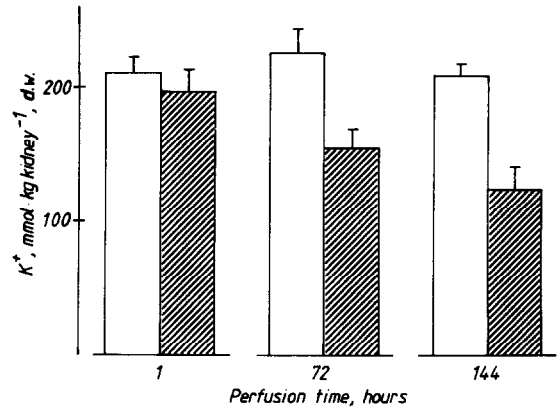

Fig. 1. Potassium content of unsliced, unincubated kidney cortical tissue (□) and tumour tissue (▨) after various times of continuous hypothermic perfusion. Mean ± S.E.M. (n = 9) (n = 7.72 hr).

Na^+-K^+ pump, in a sense reflecting tissue viability, can be deduced from measurements of steady-state levels of potassium. Figure 2 gives the potassium content in tissue slices from kidney cortex and kidney tumour after hypothermic perfusion and various time of incubation. An approximate equilibrium in K^+ distribution seemed to prevail between 60 and 120 min of incubation for both types of tissue. Concerning normal cortical tissue, this steady-state level was unchanged after 6 days of hypothermic perfusion, indicating preserved ability of the tissue for transmembrane K^+ transport *in vitro*. However, tumour tissue slices obtained from kidneys perfused for 1 hr showed a significant increase in K^+ content at the beginning of incubation ($P<0.01$). Then after prolonged hypothermic perfusion the steady-state K^+ level in incubated slices declined, indicating impaired viability of the tumour tissue.

The water content of unsliced, unincubated and sliced, incubated specimens from kidney

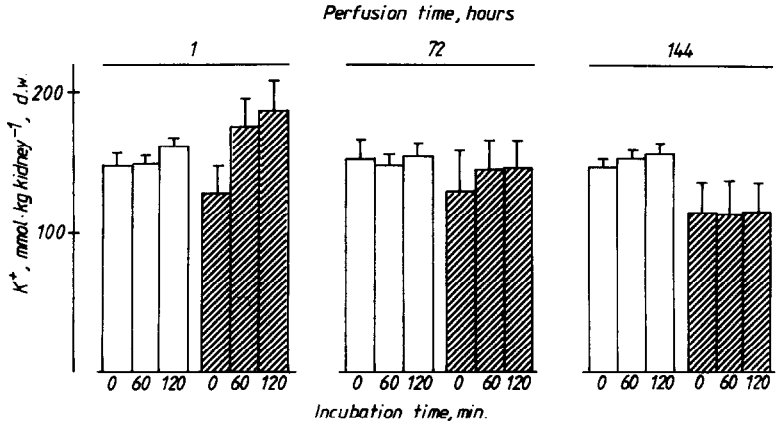

Fig. 2. Potassium content of slices from kidney cortical tissue (▨) and tumour tissue (□) after continuous hypothermic perfusion and various times of incubation. Mean ± S.E.M. (n = 9) (n = 7.72 hr).

cortical tissue and kidney tumour tissue after hypothermic perfusion is given in Table 1. The water content was lower in tumour tissue than that of cortical tissue after all three periods of hypothermic perfusion. Incubated slices had a reasonably constant water content between 60 and 120 min of incubation.

Perfusate distribution

Pressure flow characteristics for the whole preparation are presented in Table 2. No remarkable change in resistance occurred over the period studied.

Regional perfusion data derived from activity measurements in defined pieces of tissue are presented in Fig. 3. Since no absolute flow data were available, flow in the cortex was arbitrarily nominated 1.

Measured tumour perfusion was initially approximately 60% of that of cortex. After 6 days of perfusion this percentage had decreased to approximately 30%. Initial perfusion of the cortex was homogeneous and in pronounced contrast to the heterogeneity observed in tumour tissue. This is also illustrated by the autoradiograms (Fig. 4). When the perfusion pattern of the autoradiograms was compared to the vascularity seen in the radiographs there was a congruence between well-vascularized and perfused areas.

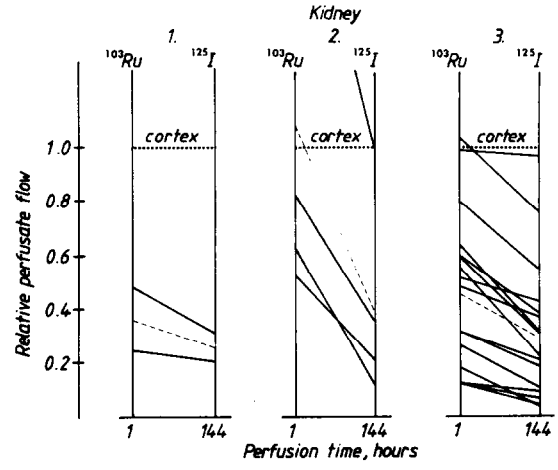

Fig. 3. Relative perfusate flow of human kidney cortex and tumour during continuous hypothermic perfusion. Perfusion of the cortex was arbitrarily nominated 1 and the flow of tumour samples are a fraction of these. The mean tumour flow (---) in the three kidneys are given.

Table 1. Water content of unsliced, unincubated and sliced, incubated specimens from kidney cortical tissue and kidney tumour tissue after continuous hypothermic perfusion. Mean ± S.E.M. (n = 9) (n = 7.72 hr)

Tissue preparation	Incubation time (min)	Water content (ml/100 g wet wt) Cortical tissue perfusion time (hr) 1	72	144	Tumour tissue perfusion time (hr) 1	72	144
Unsliced, unincubated tissue		82.6 ± 0.7	82.5 ± 0.6	82.4 ± 0.6	77.1 ± 1.5	79.7 ± 1.3	78.3 ± 1.1
Sliced, incubated tissue	0	83.7 ± 0.6	84.3 ± 0.5	83.8 ± 0.4	80.4 ± 1.6	79.8 ± 1.6	80.5 ± 1.3
	60	82.1 ± 0.7	83.1 ± 0.6	83.3 ± 0.5	78.2 ± 2.1	76.5 ± 2.3	80.4 ± 1.1
	120	83.0 ± 0.4	82.2 ± 0.7	82.6 ± 0.5	77.6 ± 1.8	76.4 ± 2.2	79.5 ± 1.1

Table 2. Pressure flow characteristics of human kidney with carcinoma after 1 and 144 hr of continuous hypothermic perfusion (n = 3)

Kidney No.	Weight (g)	Perfusion time (hr)	Flow (ml/min)	Mean arterial pressure (mm Hg)	Flow resistance (PRU_{100}), (mm Hg min 100g tissue/ml)
1	164	1	290	47	0.27
		144	250	47	0.31
2	208	1	335	43	0.27
		144	325	40	0.26
3	317	1	385	28	0.23
		144	430	32	0.24

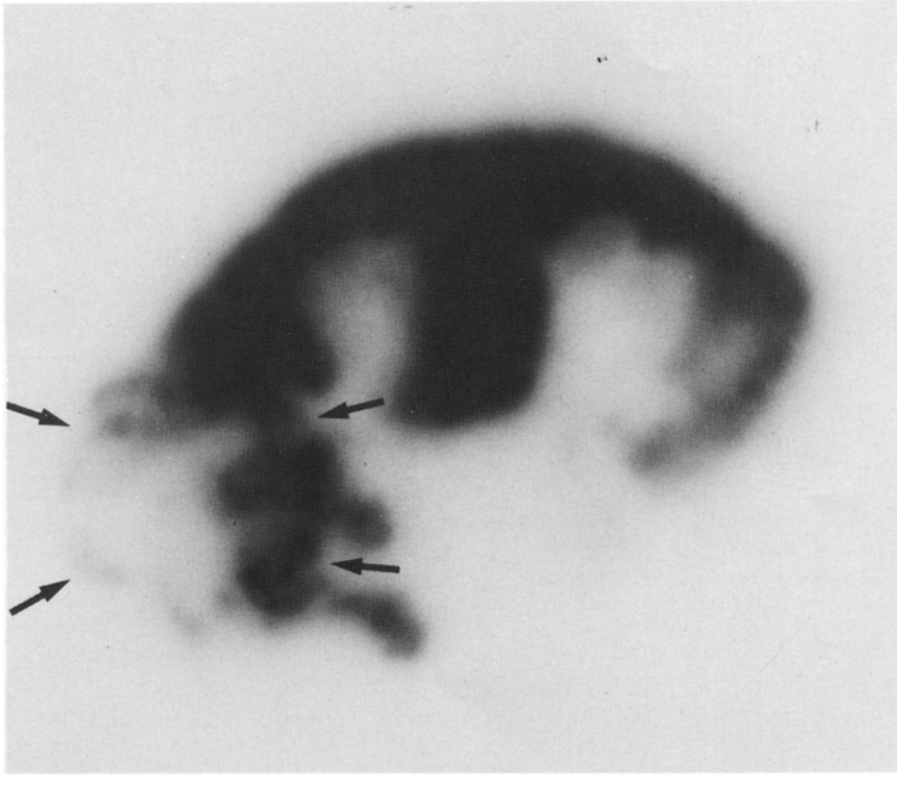

Fig. 4. Autoradiogram from a hypothermically perfused tumour-involved (→) kidney (No. 1).

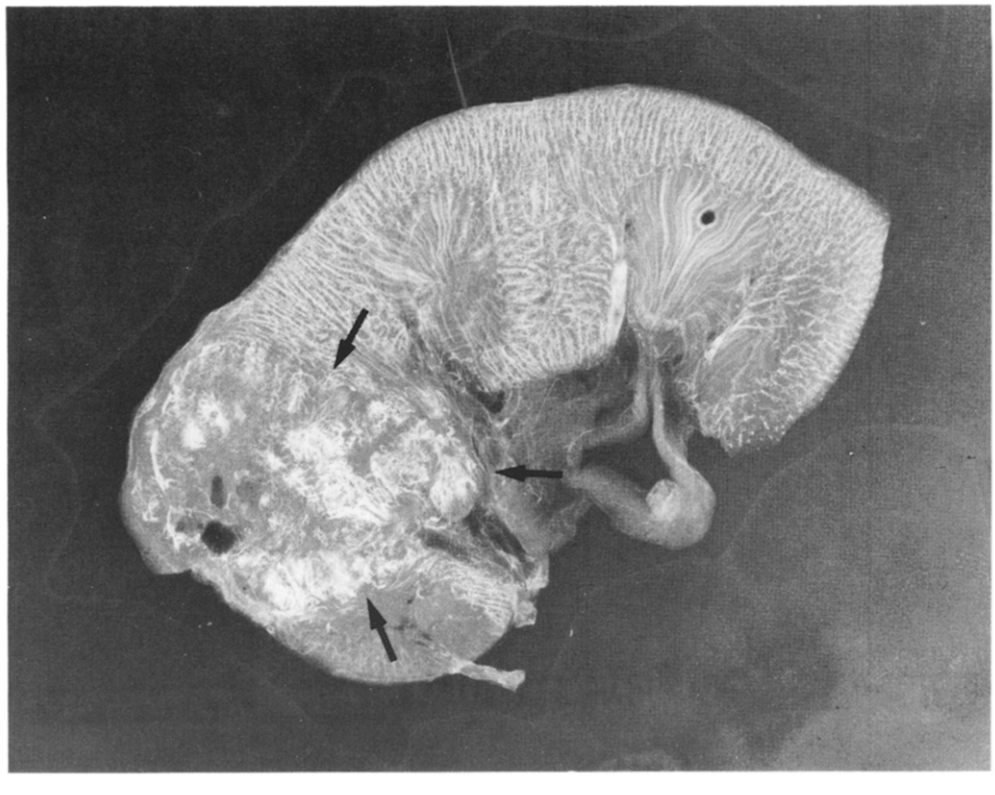

Fig. 5. The vascular network visualized by contrast perfusion of a tumour-involved (→) kidney (No. 1).

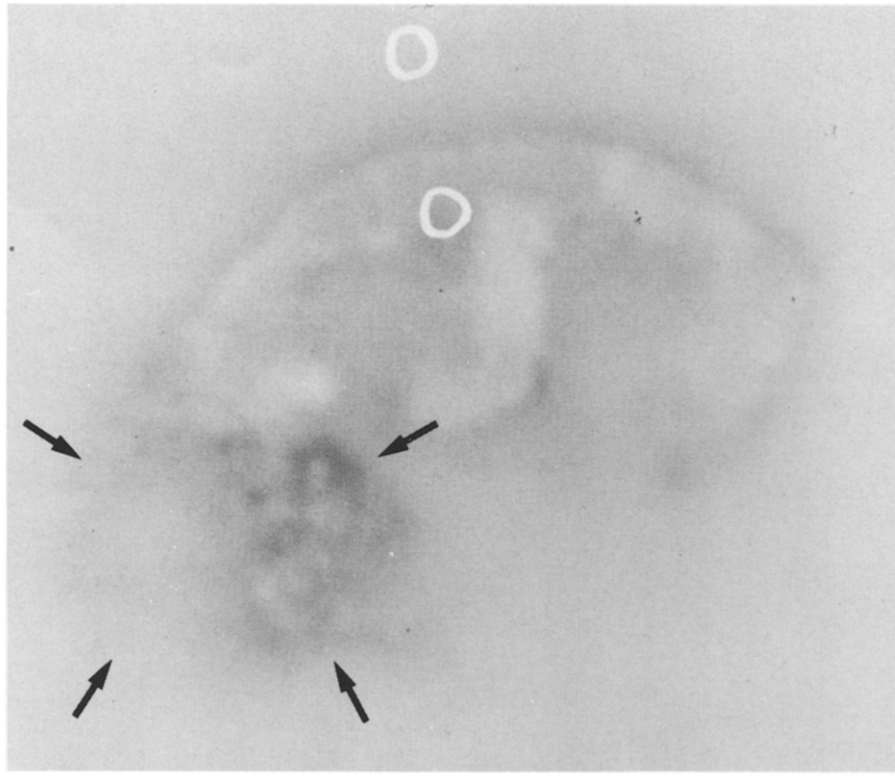

Fig. 6. *'Supersubtraction' autoradiogram from a hypothermically perfused, tumour-involved (→) kidney (No.* 1).

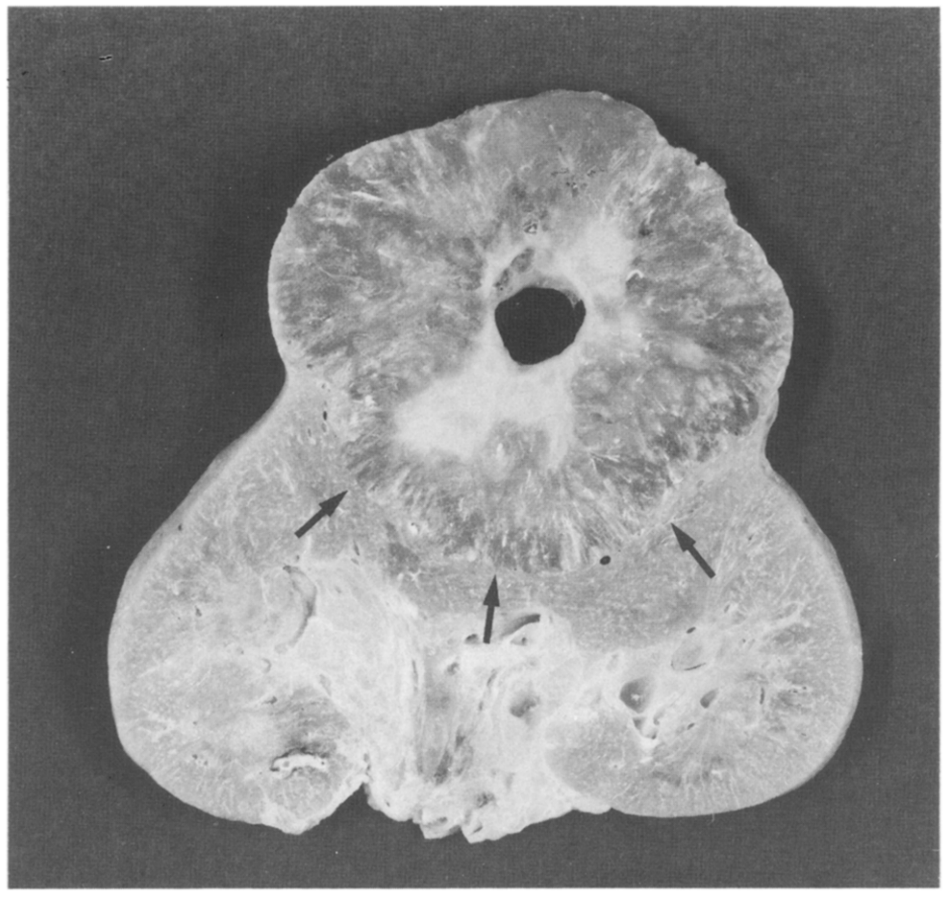

Fig. 7(*a*).

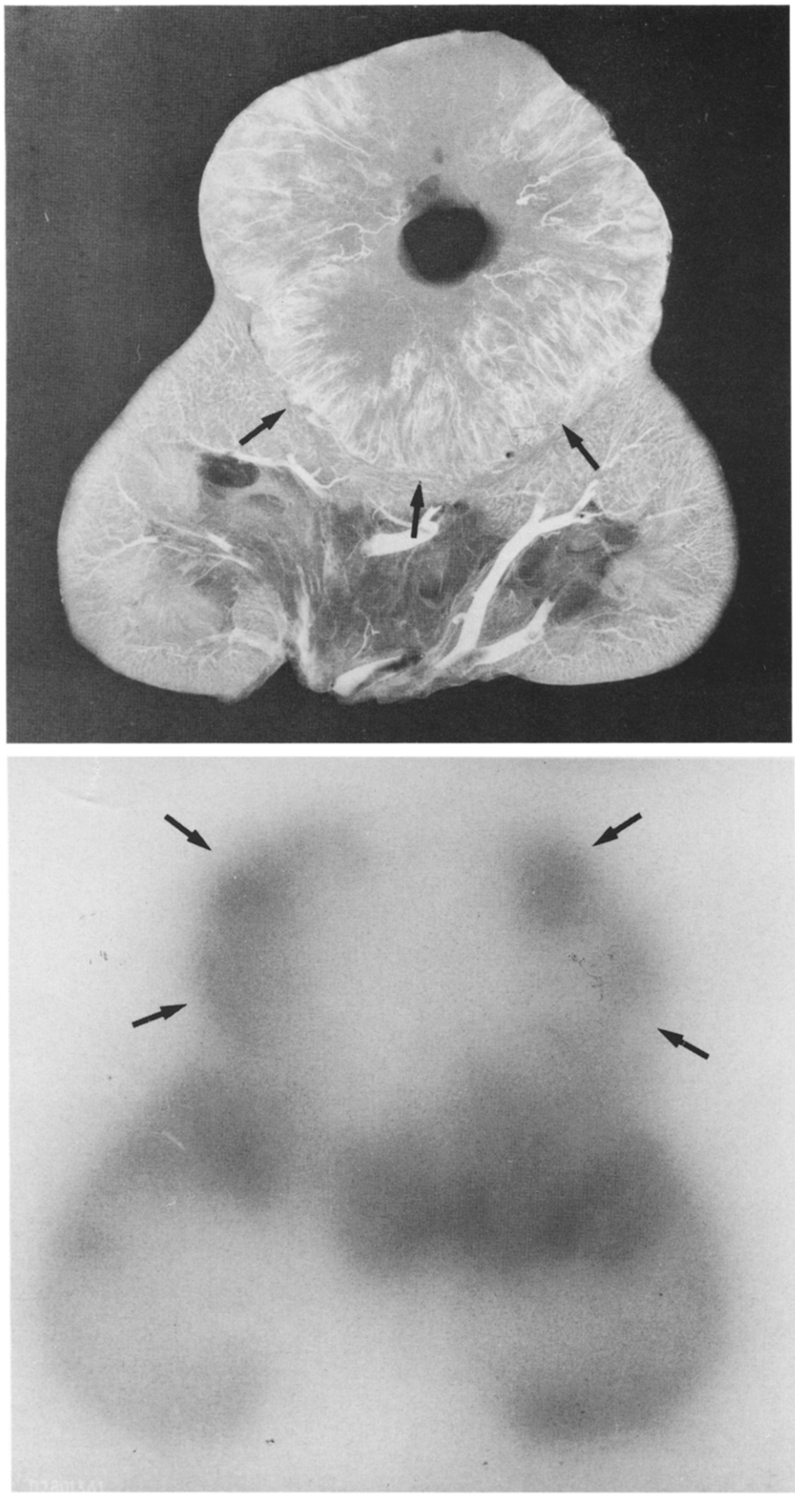

Fig. 7 (a–c). Median section of a hypothermically perfused, tumour-involved (→) kidney (No. 3); macroscopic (a), angiographic (b) and autoradiographic (c) pictures.

The regular vascular network of normal kidney was well-visualized and contrasted to the tuftlike, cotton-wool appearance of the tumour vessels (Fig. 5). The decrease in tumour perfusion relative to cortex after 6 days of perfusion, illustrated in Fig. 3, could also be demonstrated by 'super-subtraction' autoradiography (Fig. 6). In one of the kidneys (No. 3) with a rather large tumour (diameter 7 cm) a pronounced gradient from periphery to centre was seen macroscopically as well as angiographically and autoradiographically (Fig. 7a–c).

No radioactivity could be recovered in the perfusion set.

DISCUSSION

The results of the present study indicate that 6 days of hypothermic perfusion caused a more pronounced decrease in viability and perfusate flow in the tumour tissue than in the normal renal parenchyma. Consequently, the potassium content decreased in the tumour tissue while it was essentially unchanged in the cortical tissue. The steady-state levels of potassium in incubated slices, reflecting the transmembrane K^+ influx [9, 10], was unchanged after 6 days of perfusion in normal cortical tissue while a significant reduction was observed in the tumour tissue. The relative perfusate flow was already lower in the tumour tissue at the start of the perfusion than in the normal renal parenchyma (60%) and was further decreased after 6 days of perfusion (30%).

The regional perfusate flow was only determined in relation to flow in the cortex in the present study. However, since the total perfusate flow was not altered during 6 days of perfusion, the present data indicate that the absolute perfusate flow was decreased in the tumour tissue.

In this study the microspheres were injected into the afferent tubings only 5–10 cm from the kidney. It can be argued that, when microspheres are used, they should be injected far enough from the organ studied to avoid an uneven distribution of spheres in the perfusate. However, the results of this study, with an even distribution of the spheres in the parenchyma, contradict the presence of this error, which may be explained by the high flow rate in the system.

After 1 hr of hypothermic perfusion an increase was observed in the potassium content of tumour tissue slices before a steady state was achieved. This might be explained by a reversible depression of the Na^+–K^+ pump by hypoxia during the first hour of perfusion. The absence of the same phenomenon in the cortical tissue may be explained by a more homogeneous perfusate flow and, consequently, better oxygenation. This interpretation is supported by the results from the microsphere studies. After 72 and 144 hr of perfusion the capacity of the tumour tissue to respond to incubation by activating the Na^+–K^+ pump was successively reduced.

Arteriovenous shunts have been suggested to exist in tumour tissue by previous workers [11, 12]. In the present study no radioactivity could be recovered in the perfusion system after passage through the kidney. Though based on few experiments, this observation is not consistent with large anatomic ateriovenous shunts. Furthermore, this interpretation is supported by a previous study *in vivo* using a dye dilution technique which produced no evidence for shunting of blood in tumour tissue [13].

The pathogenesis of the decreased viability of tumour tissue during continuous hypothermic perfusion may be explained in different ways. The perfusion of the tumour tissue was already, after 1 hr of hypothermic perfusion, only 60% of that of the normal parenchyma, an observation which is in accordance with previous observations in cancer-involved human kidneys *in vivo* as expressed by blood flow per volume of tissue [13]. It is therefore possible that the perfusion of the tumour tissue was insufficient in the low-pressure perfusion system used, resulting in a lack of oxygen and substrates, with a consequent decreased viability of the tumour tissue. On the other hand, this interpretation does not explain why the perfusion of the tumour progressively decreased during the hypothermic perfusion. The explanation for this observation might be sought in recently observed, abnormally high, interstitial pressure in neoplastic tissue [14] compromising the perfusion of existing vascular networks. The interstitial tissue pressure could increase further with perfusion. Despite the data on water content contradicting this concept of oedema formation, an interstitial oedema could possibly exist in parallel with a decreased intravascular space [15]. Another explanation is an increased susceptibility of the tumour tissue to hypothermia *per se*.

In conclusion, the viability of renal tumour tissue was decreased more than that of normal cortex upon hypothermic perfusion. This might be secondary to impaired perfusion of tumour tissue, and further studies on the pathogenesis are in progress in our laboratory.

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