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Functional Capacity of the Isolated Perfused Dog Kidney*

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Summary. 1. Vasoconstriction in the isolated kidney can be reduced by the use of fresh blood for perfusion. The amount used for filling the perfusion machine should not exceed that volume which passes the kidney within 3 min. Mechanical stirring and haemolysis should be reduced as much as possible.

2. Initial vasoconstriction is attenuated by addition of dilating drugs to the perfusion system (promethazine, acetylcholine).

3. After a normal period of about 1 hr, total renal blood flow increases to supranormal values as high as 6—10 ml/g · min. It is believed that this dilation is due to liberation of the Hagemann factor in heparinized blood, which activates a bradykinin like substance and to the removal of vasoconstricting factors. Medullary passage times are 40% higher than those obtained in kidneys in situ.

4. Glomerular filtration rate (Creatinine clearance) reaches values (42—86 ml/100 g · min) comparable to those obtained in kidneys *in situ*. It then decreases during the second phase of the experiment, characterized by increases in total renal blood flow, intratubular pressure, and kidney weight.

5. The isolated kidney reaches a state of water diuresis within 1 hr after transfer to the perfusion system. Urine osmolality falls to values as low as 60 mOsmols/kg with a urine volume of 4 ml/100 g · min.

6. Addition of antidiuretic hormone (0.1—1.0 units/hr) prevents water diuresis but does not maintain a normal concentrating function.

7. In most of the experiments sodium reabsorption is not impaired. Large increases of Na-rejection are observed:

a) in kidneys taken from dogs having received a high sodium diet for 3 weeks prior to isolation of the kidney.

b) after infusion of saline either into the animal $^1/_2$ hr before isolation of the kidney (500 ml) or during the perfusion experiment (150 ml).

8. Impairment of Na reabsorption coincides with increased K excretion.

9. It can be stated that the isolated perfused kidney is able to function like a normal kidney *in situ*. However, there is a progressive impairment of filtration and concentrating processes, for which oedema might be at least partly responsible.

Many attempts have been made to maintain the functional capacity of the kidney after its transfer to the oxygenator and perfusion machine. Neither renal blood flow or filtration rate, nor urine concentrating ability could be kept at normal values. Recently some of us have shown that

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vasoconstrictive material exists in red cells from which it is continuously released. When blood is stored, vaso-constrictive material builds up to an abnormally high concentration [1]. This holds true not only for storage in glass vessels but also for storage in occluded blood vessels. It therefore appears incorrect to consider a buildup of vasoactive material purely as an experimental artefact. Under normal physiological conditions an equal amount of vasoactive material is released, but due to its metabolization, elimination or inactivation by various organs of the body (lungs, liver and kidneys, as recently shown by some of us [1—3, 8, 10], its concentration never reaches a high level.

These findings suggested that more successful renal perfusion might be performed if accumulation of vasoconstrictive material were prevented or minimized. We therefore tried to fulfill three conditions in order to accomplish this goal.

1. Kidney perfusion should start as early as possible after transfer of donor blood to the perfusion machine.

2. The total amount of blood used in the perfusion system should not exceed that volume which normally flows through the kidney within 3 min. Under such conditions, continuous removal of vasoconstrictive material prevents its accumulation which would induce vasoconstriction.

3. Oxygenator and pump should produce as little mechanical stirring and haemolysis as possible. Both factors increase vasoconstriction.

Although we did not succeed in preserving the normal functions of the isolated organ for a longer period than 3 hrs, we found that within this period, the perfusion machine could at least be used as a tool in studying physiological processes.

Materials and Methods

The perfusion machine has been described elsewhere in detail [3, 7, 8]. A schematic drawing is given here (Fig. 1). The equipment includes a double cylinder oxygenator and a Dale-Schuster type pump. All dogs were under Nembutal anaesthesia (30 mg/kg i.v.). In all experiments, both kidney and blood were taken from the same donor and blood was not replaced during the experiment. The blood donors received a previous intravenous injection of heparin (1250 IU/kg). Blood was then introduced from the cannulated carotid artery into the machine immediately before the beginning of perfusion. The amount of blood used corresponded to approximately 10 times the weight of the perfused kidney. The perfusion pressure was 110 mm Hg in all experiments (unless otherwise stated).

In order to compensate for urinary excretion a continuous infusion was given at a rate of 6 ml/hour. The solution contained 2 g glucose, 2 g urea, 1.5 g potassium chloride per 100 ml Ringer solution. In addition urine was continuously replaced by equal volumes of Ringer solution. When urine was hypotonic, concentration of these solutions was reduced by one-half.

Glomerular filtration rate was measured by exogenous creatinine clearance. A priming dose of 20 mg creatinine per 100 ml blood was given at the beginning of artificial perfusion and was followed by a continuous infusion at 6 ml/hr of a 2% creatinine solution. Clearance periods lasted 20 or 30 min.

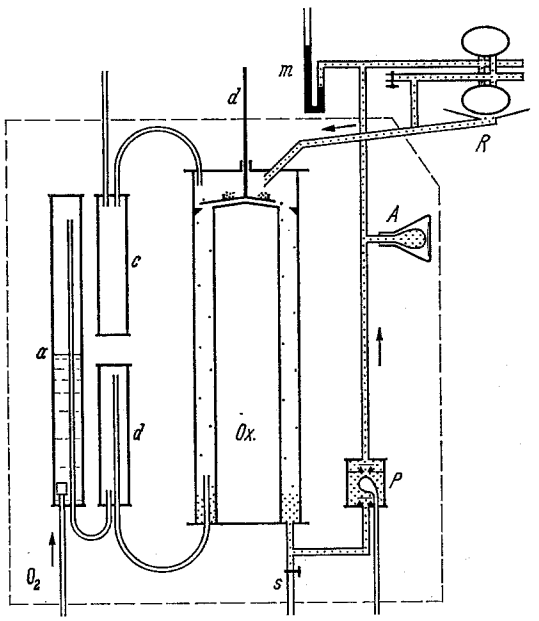

Fig. 1. Schematic drawing of the perfusion system for isolated kidneys. *Ox* Dubble-cylinder oxygenator, *d* Rotating disc., *a* Saturation of O_2 CO_2 mixture with water, *P* Dale-Schuster type pump, *A* Pulsation damper, *R* Dish holding perfused organs, *C* Condensation water trap

Total renal blood flow was measured with a stop watch and a graduated pipette. In some experiments, continuous recording was made with a rotameter.

Inner medullary blood flow was investigated using the photoelectric technique previously described by KRAMER *et al.* [4].

Intratubular pressures were measured with the micropuncture technique.

Results

1. Renal Blood Flow

A typical pattern of the course of total renal blood flow (TRBF) is given in Fig. 2a. The initial blood flow is approximately normal but within 1—2 min decreases to values far below normal levels. It then increases progressively to normal values (3.8 ml/g · min) during the first 30—60 min and to supranormal values (6 ml/g · min) during the rest of the experiment (Table 1).

The duration of the initial decrease in TRBF can be reduced somewhat by injection of dilating drugs at the beginning (acetylcholine, promethazine) (Fig. 2b). It is very difficult to avoid completely an initial decrease of TRBF. Moreover, the initial addition of vasodilating drugs does not influence the increase of TRBF during the second hour. However the final rise of TRBF to supranormal values can be reduced by the

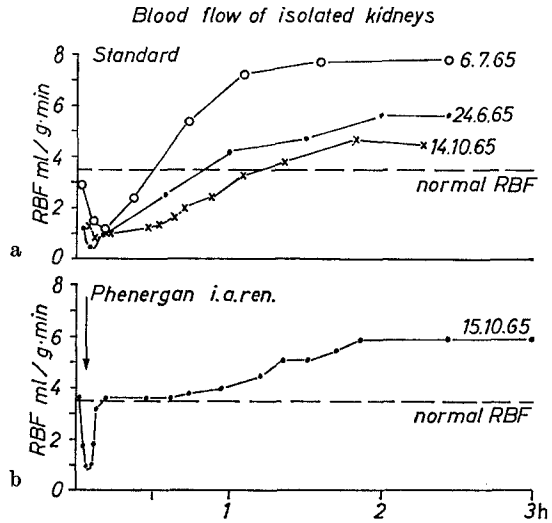

Fig. 2. a Course of total renal blood flow in experiments on 3 isolated kidneys during a period of 3 hrs. b Effect of promethazine injected into the renal artery during the initial period of vasoconstriction

Table 1. *Steadystate value of TRBF at the last phase (3rd hour) of the experiments on isolated perfused kidneys*

Experimental condition	Nr of experiments	Mean TRBF ml/100 g · min	S_D	P vz Control
Low sodium diet	7	600	± 120	Control
High sodium diet	4	700	± 190	< 0.2
Low Na diet + ADH	7	670	± 110	< 0.2
Low Na diet + SBTI	5	450	± 90	< 0.05

addition of soya-bean trypsin inhibitor (SBTI)[1] at the beginning of perfusion (1 mg/100 ml of blood), SBTI inhibiting the Hagemann factor (see below) (Table 1). The probable role of permeability factors is demonstrated by the development of oedema (see later).

In order to investigate a possible influence of the renin content of the kidney on the development of vasoconstriction a series of experiments were carried out on dogs which had been given a high sodium diet over a period of 3 weeks prior to the experiments. In some cases kidneys taken from donor dogs on high sodium diets showed a high blood flow throughout the entire experiment. The supranormal phase, however, could not be avoided.

[1] We are indebted to Dr. J. SALMON for suggesting the use of SBTI.

Infusions of ADH (vasopressin Sandoz) 0.1—1.0 units per hour, were without effect on the typical blood flow pattern.

2. *Inner Medullary Blood Flow* (Fig. 3)

In 5 experiments the mean medullary passage times (MPT) of cardiogreen were recorded by means of a medullary photometer. During the constrictive phase the passage of the dye was found to be slowed down. The MPT may have been more than 100 sec. With the recovery of total blood flow the MPT decreased to values of 33.2 sec $\pm$ 5.3, and maintained

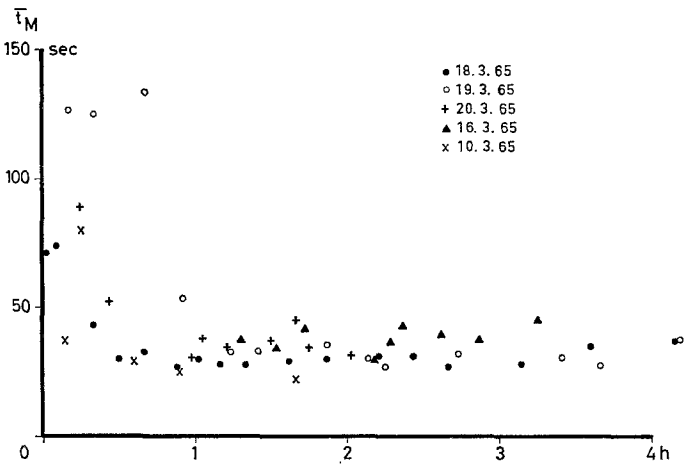

Fig. 3. Dye passage times (t_M) of the inner medulla during the course of experiments on 5 isolated kidneys

these values during the entire course of the experiments, even when TRBF exceeded normal values. However it should be noted that a value of 33.2 sec, exceeds by 40% the normal values observed in kidneys *in situ*.

3. *Intratubular Pressures*

Intratubular pressure was measured by micropuncture during the course of 4 experiments. Immediately after the start of perfusion many superficial tubules were collapsed. The pressure in the open tubules (about $^1/_3$ of the tubules in the microscopic field) was 18—21 cm water. With the recovery of blood flow during the first hour, the intratubular pressure of proximal tubules, showed a steep rise to values of 40—60 cm water. By that time, most of the superficial tubules were perfused and exhibited uniform pressure. During the course of the experiments intratubular pressure increased progressively to values up to 70 cm water (Fig. 4). In this phase of the experiments, the proximal tubules appeared

to be maximally dilated (70% increase in diameter) and the rate of tubular flow was drastically reduced. In two experiments we succeeded in measuring the pressure in distal tubules, identified by arterial lissamine green injection, during the second and third hours of perfusion. Pressure values were consistently found to be 20—30 cm lower than those of proximal tubules.

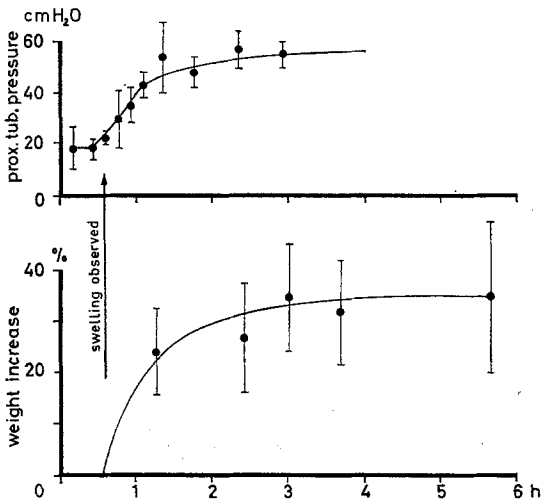

Fig. 4. Proximal tubular pressure (3 experiments) and weight increase (20 experiments) during experimental periods of 3 and 6 hrs resp.

4. Kidney Weight (Fig. 4)

There was a progressive increase in weight of perfused kidneys during the experiments. Fig. 4 gives the weight increase of decapsulated, blood-free kidneys after various durations of the experiments; the contralateral kidney was used as a reference. Since either the right or the left kidney was used for perfusion no correction for left-right weight differences had to be made. In 5 experiments the weight of the inner medullary tissue was compared with the corresponding tissue of the contralateral kidney. The increase of weight amounted to about 20% after 3 hrs of experiments. Soya-bean trypsin inhibitor (see above) had no significant effect on weight increase. It should be noted that the increase of weight and tubular pressure showed a parallel course.

5. Glomerular Filtration Rate (Creatinine Clearance)

Creatinine clearance obtained during the first 10 min of perfusion could not be considered a valid measure of GFR because of the interference of washout effects in the tubules and in the pelvis. In cases in

which vasoconstriction lasted longer than 1 hr, GFR remained below the normal level throughout the experiment. In kidneys exhibiting only a transient initial vasoconstriction, GFR during the first hour of perfusion reached values comparable to those in normal kidneys in situ (45—82 ml/100 g). During the second and third hours of perfusion, GFR showed a continuous decrease to $^1/_2$ or $^1/_3$ of maximum values. As mentioned before,

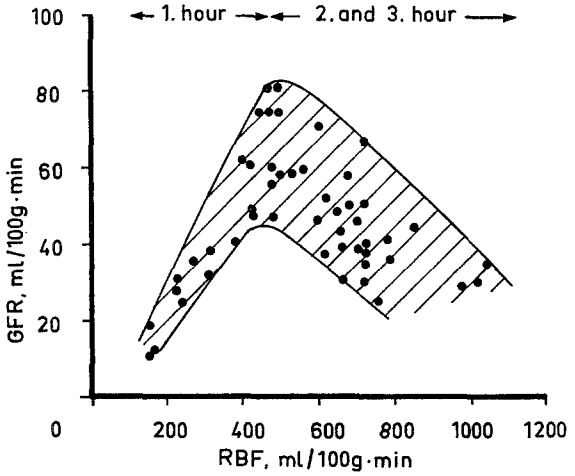

Fig. 5. Relation of GFR (creatinine clearance) and total renal blood flow (TRBF) during the course of 3 hrs experiments. Values are taken at different times as indicated

TRBF reached supranormal values during the second and third hours. In this phase the decrease in GFR coincides with the increase in TRBF (see Fig. 5). All attempts to correlate the behaviour of GFR to the diet of the donor dog were unsuccessful; neither high nor low Na^+ diet had any detectable influence on GFR in the isolated kidney. However, saline seemed to have a definite influence when it was added to the perfusion system $1^1/_2$ hr after beginning of perfusion. In 9 experiments, GFR increased from 34.2 ± 7.7 to 53.3 ± 15.1 ($P < 0.01$). However, after a period of less than 1 hr GFR decreased and reached as in the other experiments values far below normal. Both haematocrit and protein concentration fell after an infusion of saline into the perfusing blood to approximately 60% of normal values (Fig. 8).

6. Concentrating Ability

When kidneys from hydropenic dogs were transferred to the perfusion machine, the osmolality in the first urine sample dropped considerably below the last bladder urine sample (Table 2). In a few experiments,

Table 2. *U/P osmoles before and after isolation of the kidney. U/P osmoles values. 1. In bladder urine taken immediately before transfer of the kidney to their perfusion machine. 2. In the urine of the perfused kidney at various periods of the experiments. (7 experiments selected out of a series of 34 experiments which show unset of recovery of the concentrating mechanism in the first phase of the perfusion. After 3 hrs of perfusion in most of the experiments hypotonic urine is excreted except if ADH is added to the perfusion system)*

Bladder urine		Perfused kidney Start—20	20—40	40—60	180 min
15.6	1.90	1.85	2.15	0.47	0.75
22.6	1.90	2.26	2.43	1.83	0.49
15.7	5.06	2.02	2.10	1.96	0.45
30.9_1	4.27	2.19	2.47	1.47	0.50
30.9_2	3.97	1.41	1.81	1.93	0.05
14.9_2*	6.90	1.62	1.72	1.81	0.93
16.9*	3.67	2.04	2.50	1.95	1.15

* With ADH infusion.

Table 3. *U/P osmoles, GFR, urine volume and sodium rejection in 6 experiments on perfused kidneys during the phase of fully developed water diuresis*

	U/P osmoles	GFR ml/100 g · min	U vol. ml/100 g · min	Na rejection %
6. 7. 65	0.34	48	3.85	0.38
13. 7. 65	0.36	44	2.73	0.56
15. 7. 65	0.31	50	3.60	0.82
9. 7. 65	0.36	90	2.72	0.64
22. 6. 65	0.23	52	2.76	0.31
17. 6. 65	0.37	45	4.14	0.38
Mean	0.33	55	3.30	0.67

Table 4. *U/P osmoles in ADH-experiments. U/P osmoles of bladder urine before isolation of the kidney, minimal and final values in the urine of the perfused kidney in the presence of ADH, 0.1 unit/h were infused into the perfusing system throughout the experiment. In the last experiment (*) ADH was given $1^1/_2$ hr after the beginning of perfusion, at the moment U/P osmoles had fallen to 0.46*

Kidney in situ	Bladder urine	Isolated kidney During ADH-Infusion Minimum value	Final value (3rd hr)
$20.\ 9.\ 65_2$	6.0	1.30	1.30
$14.\ 9.\ 65_1$	6.3	1.10	1.10
$14.\ 9.\ 65_1$	6.9	0.9	0.93
$16.\ 9.\ 65_1$	3.7	1.14	1.00
13. 9. 65*	1.78	1.0	1.09

osmolality rose again in the first hour, although U/P osmoles never reached values higher than 2.5. After the first hour, osmolality fell to values as low as 60 mOsm/kg i.e., in terms of U/P osmoles to about 0.2 at plasma levels of 300 mOsm. This indicates a full development of water diuresis. Urine flow on only one of the 7 evaluated experiments was comparable to a diabetic insipidus state (13.2 ml/100 g · min), the mean urine flow in the other six experiments did not exceed 3.53 ml/100 g · min (Table 3). In the third hour, urine osmolality rose again towards isotonicity, U/P osmoles however did not increase because of increasing plasma osmolality (see later).

A continuous addition of ADH did not prevent a progressive decrease in urine concentration. However, urine osmolality never became hypotonic. Doses of 0.1—0.5 IU per hour, were usually required to prevent water diuresis (Table 4). Higher infusion rates did not improve the response significantly.

ADH added during the period of water diuresis caused a slight drop in urine flow with only moderate hypertonicity, In spite of the high amounts of ADH used, no vasoconstriction occurred.

7. Sodium Excretion

Excretion of sodium in the isolated kidney depended on the salt content of the diet previously given to the donor dog. Kidneys of dogs having received a high sodium diet for 3 weeks (15 mEq/kg day) excreted, even in an isolated state, large amounts of Na^+ during the first hour of the experiment. Na rejection reached values of 3.3%. Within the second hour Na excretion neared the low level found in kidneys taken from dogs which had received a low sodium diet; Na rejection was about 0.5% (Table 5). During the third hour, Na rejection may rise.

A high sodium excretion was also observed when 500 ml saline was infused 30 min prior to the experiment into a dog having received a normal Na diet (Table 5). Even higher excretion was induced when isotonic saline (150 ml) was infused into the machine during perfusion (Fig. 8).

During water diuresis the replacement of fluid loss was critical. Because of the low resistance of dog erythrocytes, this replacement could not be made with solutions having an osmolality lower than half isotonic value. However, in all cases where Na excretion was low 50% diluted Ringer used for replacement resulted in a rather large increase of plasma Na concentration up to 190 mEq/l. Only in experiments with high Na excretion could the plasma Na level be kept constant. This was observed in 10 experiments in which Na excretion was enhanced by saline infusion either prior to or during the experiments. Under such conditions, plasma Na concentration varied not more than 5 mEq/l.

Table 5. *Sodium handling in isolated perfused kidneys*

	GFR (ml/100 g · min)	Na Load	Na Output	Na-rejection %
		Low sodium diet (2 hrs of exp.)		
20. 9.$_1$	60	9.100 μEq/min	64 μEq/min	0.7
5. 10.	32	5.100	2.5	0.05
20. 9.$_2$	48	5.700	17.0	0.3
30. 9.	40	6.400	4.5	0.07
13. 7.	40	5.600	25.0	0.45
6. 7.	52	7.800	35.0	0.45
15. 7.	55	8.500	44.0	0.52
Mean	48	6.900	33.0	0.47
		High sodium diet (1st hr of exp.)		
15. 6.	85	13.000	408	3.2
17. 6.	38	5.300	90	1.7
22. 6.	46	6.700	120	1.8
14. 9.	60	9.100	297	3.3
Mean	57.25	8.525	228.75	2.5
		High sodium diet (2nd hr of exp.)		
17. 6.	45	6.900	95	1.4
22. 6.	50	7.700	31	0.4
14. 9.	50	10.000	70	0.7
Mean	48	8.200	65	0.8
		Saline infusion (500 ml) $^1/_2$ hr before isolation of kidney (2 hrs of exp.)		
18. 10.	35	5.950	164	2.8
6. 10.$_2$	56	9.000	200	2.2
15. 10.	36	7.200	325	5.4
21. 9.	36	5.100	208	4.1
14. 10.	40	7.100	230	4.5
13. 10.	50.1	7.000	210	3.0
12. 10.	48.5	7.900	262	3.3
Mean	42.8	7.000	230	3.3
		Saline infusion (150 ml) after $1^1/_2$ hr of isolation of the kidney ($1^1/_2$ hr)		
25. 5. 66	45	7.200	590	8.2
8. 4.	35	5.500	306	5.6
17. 6.	50	7.750	400	5.1
15. 6.	48	6.720	850	12.6
6. 7.	48	7.920	468	5.9
1. 4.	41	6.750	156	2.3
Mean	44.5	6.973	461	6.6

In some experiments fluid loss was replaced by half isotonic glucose solution which resulted in a relatively constant plasma Na concentration. However this entailed the disadvantage of osmotic diuresis.

8. Potassium Excretion

At normal plasma sodium levels, potassium excretion was lower than potassium input (20 μEq/min) (see Table 6). When GFR decreased, as it did in the second phase of the experiments, there was a simultaneous decrease in potassium excretion (Fig. 7). On the other hand potassium excretion increased when plasma sodium concentration rose to values higher than 180 mEq/l (Fig. 6). However, kidneys with high Na rejection due to saline infusion did not exhibit similar increases in K output (see Fig. 8).

Table 6. *Potassium excretion during 3rd hr of experiments*

No. of exp.	Na Rej. %	Potassium excretion (μEq/100 g · min)	GFR (ml/100 g · min)
		Hypernatremia (180—200 mEq/l plasma)	
8	5.5	83	33
		Normonatremia (140—160 mEq/l plasma)	
9	1.0	30	32
		Saline infusion $1^1/_2$ hr after beginning of exp. (normonatremia)	
6	5.0	34	36.5

The higher K excretion associated with hypernatremia (Table 6) could be expected, since in these experiments, high diuresis required replacement of fluid with large amounts of potassium containing Ringer solution.

For comparison, the lack of any effect on K excretion when saline was infused during the second period of the experiment is shown. Under these conditions, plasma Na level was not significantly modified.

A progressive increase in plasma potassium was usually observed.

9. Urea

a) *Urea excretion* varied widely with GFR and urine flow. Under conditions of high urine flow its clearance reached values approximately 60% of the creatinine clearance (Fig. 6).

b) *Plasma-urea* concentration varied within the limits of 2.5—6.6 mMol/l. The low values were found during water diuresis, when urea output was high and replacements (see methods) not properly adjusted.

The following graphs present a synopsis of the aforementioned variables (water diuresis, antidiuresis, and saline diuresis) in three typical experiments.

10. Water Diuresis (Fig. 6)

Water diuresis is bound to appear when ADH of the donor blood is eliminated. The figure shows that water diuresis developed within 1 hr

after transfer of the kidney to the perfusion machine. TRBF reached a normal value of 380 ml/100 g · min after 30 min. GFR climbed from a low value of about 40 to a normal value of above 60 ml/100 g · min, which it maintained over 42 min.

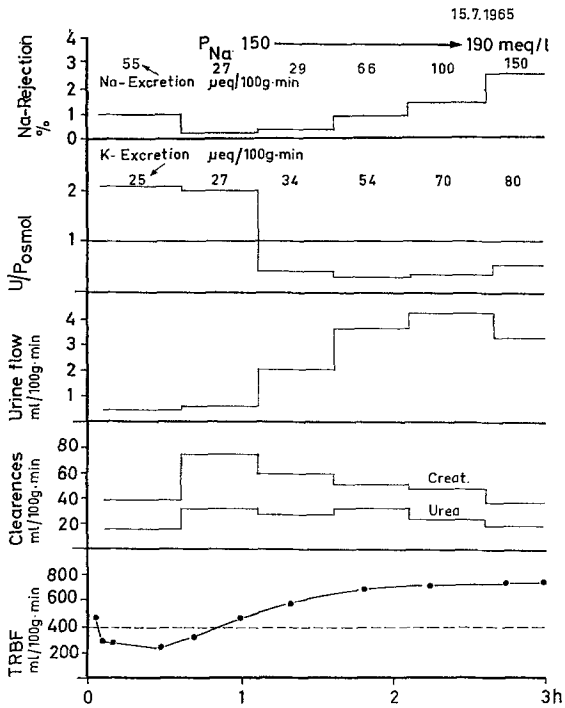

Fig. 6. Isolated perfused kidney in water diuresis, kidney (37 g) taken from a dog under low Na diet, perfusion volume 500 ml

In the next 2 hrs GFR fell to values approximately 60% of normal. During this period a full water diuresis developed. Urine flow reached values of about 4 ml/min and the U/P osmoles fell to 0.25. In the second and third hours of the experiment, Na rejection increased progressively to a value of 2.9% of the Na load. In this period plasma sodium had increased to 191 mEq/l. In the first hour, potassium excretion was 25 µEq/100 g · min.

During the second hour of perfusion potassium excretion increased.

Urea clearance was approximately 40—60% of creatinine clearance depending on GFR and urine flow.

11. Antidiuretic State of an Isolated Kidney (Fig. 7)

With continuous infusion of ADH it was possible to prevent water diuresis. Fig. 7 shows that at the start of perfusion U/P osmol was 2.25,

and then decreased slowly over the whole period of the 3 hrs, leveling off at a value somewhat above 1. Urine flow was extremely low, maintaining a rate of about 1% of GFR. Na excretion and potassium excretion were also very low. In order to keep the oedema within tolerable limits perfusion pressure was reduced to 90 mm Hg. This had, however, very little effect; kidney weight increased about 30%.

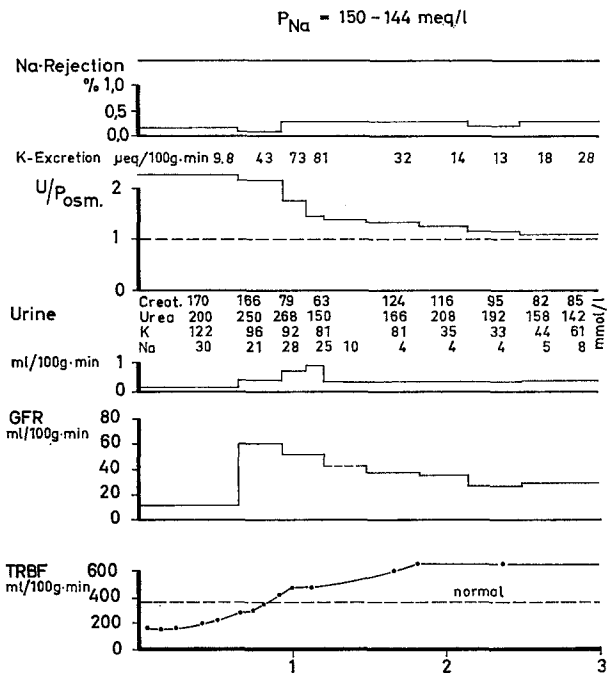

Fig. 7. Isolated kidney perfused under continuous infusion of ADH (0.1 unit/hr)

12. Saline Diuresis (Fig. 8)

The third type of experiment (Fig. 8) demonstrates the effect of 150 ml saline injected into the perfusion machine 1 hr and 10 min after the start of perfusion. As no ADH was added, water diuresis was expected to appear at that time. As found in all 10 experiments with saline infusion, GFR increased but a subsequent decrease after less than 1 hr could not be prevented. Saline infusion had a striking effect on Na excretion. In contrast to usual water diuresis, sodium output was very high and in balance with input. Fluid loss due to urine flow was replaced after each clearance period by 50% saline. This explains the fairly constant plasma sodium concentration (155 mEq/l) throughout the experiment.

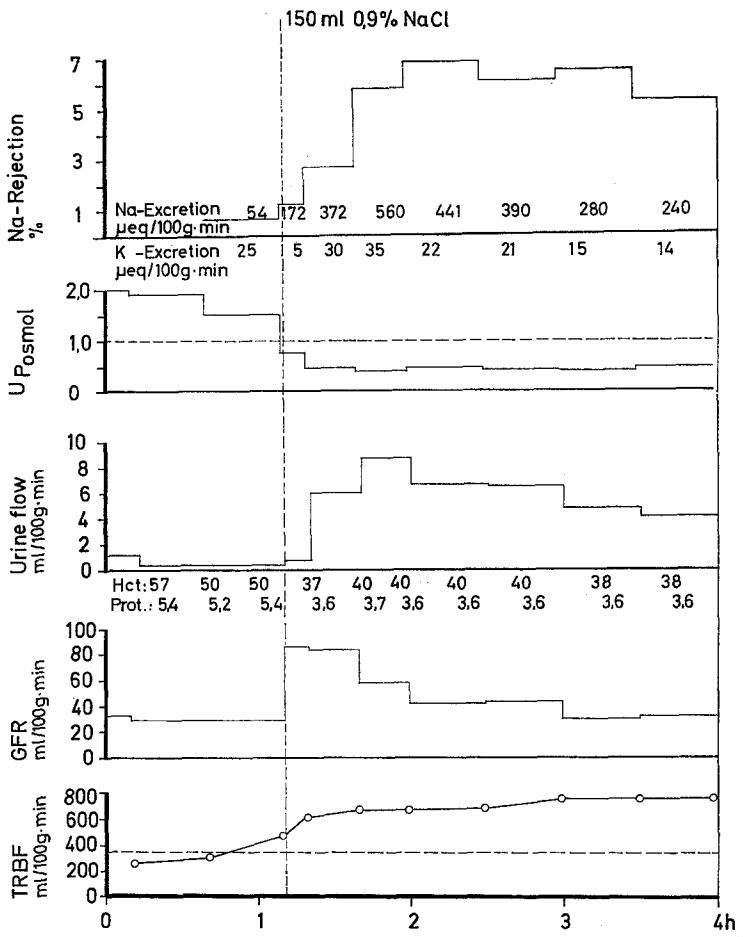

Fig. 8. Isolated perfused kidney taken from a dog under low Na-diet, effect of 150 ml saline added to the perfusion fluid

Discussion

1. Total Renal Blood Flow, Intratubular Pressures, Glomerular Filtration Rate and Kidney Weight

The initial phase of reduced TRBF (see Fig. 2a) can be related to the accumulation of vasoconstrictive material which developed during the transfer of donor blood to the perfusion machine. Although the transfer is completed within 3—4 min such an accumulation cannot be avoided. It is known from previous work [12] that accumulation is detectable after 5 min of storage at 37° C. The interval of time between the introduction of fresh blood into the machine and the beginning of kidney perfusion

should not exceed 5 min. Within that time, accumulation is not critical. A longer interval induces strong and sometimes irreversible constriction. The small amount of vasoconstrictive material built up during 5 min is removed by the kidney itself within 20—60 min (see Fig. 2a). Short initial vasoconstriction can be reduced by addition of vasodilating drugs. Under our experimental conditions, promethazine (Phenergan Specia) (Fig. 2b), an antihistaminic drug, had been added with the hope that oedema could be reduced; this did not prove successful, but Phenergan exhibited satisfactory vasodilating properties during the required time.

The final increase of TRBF to supranormal values could be the consequence of at least two factors.

1. Progressive removal of remaining vasoactive factors by the kidney, removal becoming faster than liberation in the blood. Here the possibility should be considered that the vasoconstrictive material may be normally present in the blood, its amount depending on an equilibrium between liberation and inactivation. This presents the problem of its physiological role [8].

2. Activation of dilating bradykinin-like peptides on the large wettable surfaces of the oxygenator. Oedema does not seem to be due solely to an accumulation of interstitial fluid. Because of the high proximal tubular pressures the diameter of the tubules is widened by approximately 70%. Excess intratubular fluid accounts for 60 p. 100 of total weight increase.

The rise of proximal tubular pressure during the second phase may be explained by two factors:

a) Dilation of Renal Vessels, Including Efferent Arterioles. Dilation is indicated by the fact that renal blood flow at constant perfusion pressure exceeds normal values by 50 or even 100%. Since the main resistance is in the efferent arterioles, these are bound to be dilated, which would lead to high peritubular and intratubular pressures and to oedema.

b) Increase of Flow Resistance in Henle's Loops. This is derived from the fact that tubular pressure is 20—30 cm H_2O lower than proximal. These high values indicate also an increased resistance in collecting ducts. Oedema in renal medulla, expected of the same order of magnitude as in the cortex, probably compresses tubular as well as vascular structures.

This is in agreement with the existence of higher medullary passage times than found under normal conditions.

However, it must be pointed out that distal tubular pressures could be measured in only two experiments. Further experiments are necessary, including measurements of tubular flow, in order to permit calculations of the change in resistance throughout the nephron.

It is known that heparinized blood develops two factors, a dilating bradykinin-like substance and a permeability promoting principle. Both

are due to liberation of the Hageman factor which is activated on wettable rough surfaces (MARGOLIS [6]). The development of this factor can be inhibited by Soya-bean trypsin inhibitor (RATNOFF and MILES [12]). Our findings that addition of this inhibitor diminishes the increase of supranormal blood flow (Table 1) suggests the probability that such a mechanism is involved. However, the impossibility of preventing oedema by using SBTI is not elucidated.

The probable sequence of events may be the following: after removal of vasoconstrictive material in the perfusing blood by renal activities, blood flow and GFR recover to almost normal values. The development of vasodilating and permeability factors in the heparinized blood diminishes pre- and postglomerular resistances and promotes oedema. Thereby tubular pressures increase. The oedema of medullary tissue leads to a reduced GFR to a final level of about $^1/_3$ of normal.

The transient increase of GFR after addition of saline to the perfusion fluid may be due to an increased effective filtration pressure because of reduced haematocrit and reduced oncotic pressure of plasma proteins.

2. Concentrating Ability

It is known from experiments *in situ* that manipulation of kidneys and interruption of blood flow for several minutes reduce urine osmolality. Whereas kidneys *in situ* recover total concentrating ability within 1 or 2 hrs, the isolated organ, after a very short period of normal behavior loses its capacity completely, even when ADH is added. This progressive decrease in urine osmolality may be caused by a synchronous fall of GFR, a causal relationship which is known to exist from experiments on kidneys *in situ*. We may assume that urine concentrating ability is further impaired by the medullary oedema, since the building up of a concentration gradient in the inner zone depends mainly on exchange diffusion (probably of urea). Moreover, as a normal medullary blood flow is necessary to build up the concentration gradient, a reduced flow, such as found in our experiments, would prevent proper concentrating activity.

3. Sodium Excretion

The small amount of sodium excreted by the isolated kidney indicates that Na transport system along the nephron is working well. However, the falling Na-load during the course of the experiment may obscure a slight failure of Na-reabsorption (see SELKURT [13]). It is of interest that the isolated kidney, when taken from a dog kept on high Na diet, maintains a high Na-excretion. The finding of a strongly diminished excretion in the second hour of the experiment supports the assumption that a natriuretic substance is produced in dogs with high sodium diet and subsequently eliminated. It is also possible, however, that the mechanism resides within the tubular cell.

In favor of this is the high sodium excretion which follows saline infusion after the isolated kidney has reached a steady state with regard to sodium rejection. Since these experiments were performed on kidneys taken from dogs which had been on low Na diets, a possible natriuretic factor formed in the animal before the experiment started can be excluded. The impairment of active Na-transport of renal tubules indicated by high Na rejection in kidneys taken from dogs with low salt diet is a rare event in a 3 hrs experiment provided plasma sodium is normal.

4. Potassium Excretion

Since in the standard procedure 20 μEq/min of potassium were infused into the system it cannot be decided whether the control of K excretion was accomplished by active transport processes of the kidney involving K and Na or was due to K-loss from damaged tubular cells. The excess K excretion in the hypernatremic state is in agreement with micropuncture data of MALNIC *et al.* [6]. However, our experimental approach sheds no light on their proposed explanation that the K-loss is due to an increased electrical gradient in the distal nephron resulting from higher Na-transport.

Bibliographie

1. CUYPERS, Y., G. KÖVER et A. NIZET: Arch. int. Physiol. **68**, 859 (1960).
2. — — — Arch. int. Physiol. **69**, 213 (1961).
3. — A. NIZET, and A. BAERTEN: Arch. int. Physiol. **72**, 245 (1964).
4. KRAMER, K., K. THURAU u. P. DEETJEN: Pflügers Arch. ges. Physiol. **270**, 251 (1960).
5. MALNIC, G., R. M. KLOSE, and G. GIEBISCH: Amer. J. Physiol. **206**, 674 (1964).
6. MARGOLIS, J.: Nature (Lond.) **181**, 635 (1958).
7. NIZET, A.: Bull. Acad. roy. Mad. Belg. 7è série, III, 283 (1963).
8. —, et Y. CUYPERS: Arch. int. Physiol. **65**, 642 (1957).
9. — — et L. MASSILON: C. R. Soc. Biol. (Paris) **151**, 1982 (1957).
10. — — — C. R. Soc. Biol. (Paris) **153**, 620 (1959).
11. — — — et S. LAMBERT: Arch. int. Physiol. **65**, 568 (1957).
12. RATNOFF, O. D., and A. A. MILES: Brit. J. exp. Path. **45**, 328 (1964).
13. SELKURT, E. W.: III Conf. Joshia Macy Jr Fondation, p. 103 (1951).

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