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Determination of the Glomerular Filtration Rate (GFR): Methodological Problems, Age-Dependence, Consequences of Various Surgical Interventions, and the Influence of Different Drugs and Toxic Substances

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Summary

Determinations of renal clearance of fluorescein isothiocyanate (FITC)-inulin were used for assessing the glomerular filtration rate (GFR) in rats and to characterize factors influencing the glomerular filtration capacity. In anesthetized rats, GFR develops after birth up to day 30. Thereafter, GFR remains relatively constant for up to 3 months of age and drops continuously until the 8th month. GFR can be determined *in utero*, already one day before birth, however, only at a very low level. It increases significantly on the first day of life. Even at this time the effect of furosemide on GFR can be proven. After reduction of renal mass, GFR is decreased in dependence on the extent of kidney tissue removal. However, within 2 days after unilateral nephrectomy (NX) or one week after 5/6 NX, GFR reaches values about 3/4 of the controls with two intact kidneys. Furthermore, the compensation of GFR after renal ischemia reaches 80 % of baseline values after one week. On the other hand, GFR is enhanced after bile duct ligation as a model of hepato-renal failure. It has been shown in previous experiments that pretreatment with hormones can stimulate renal tubular transport processes. Pretreatment with dexamethasone or triiodothyronine after 5/6 NX improves glomerular filtration capacity whereas in animals with ligated bile ducts dexamethasone seems to prevent the increase in GFR. After subchronic treatment with epidermal growth factor (EGF) GFR is significantly reduced. A continuous infusion of amino acids does not change GFR in the controls but enhances the filtration capacity in EGF-treated rats. But immediately after bolus injection of amino acids GFR also increases significantly in the controls. Diuretics such as furosemide, most nephrotoxic agents (cyclosporine A [CsA], heavy metals) and imidazole reduce the GFR significantly. Diltiazem reported to act nephroprotectively in CsA nephrotoxicity in human beings was without beneficial effect in rats. This could be due to species differences in GFR because the rat is one of the species with the highest glomerular filtration capacity.

Key words

Kidney • Nephrectomy • Bile duct ligation • Renal ischemia • Triiodothyronine • Dexamethasone • Epidermal growth factor • Postnatal development • Age • Inulin • Amino acid load • Furosemide • Cyclosporine A • Diltiazem • Heavy metal nephrotoxicity • Rat

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Introduction

Changes in GFR and in the clearance of creatinine are often the first signs of renal failure in patients. In order to characterize kidney functions and to clarify specific problems of renal function such as estimation of filtration fractions of electrolytes and amino acids, information about GFR is essential. Many experimental approaches to determine GFR have been used in the past, nevertheless, some methodological difficulties do still exist. Besides exactly defined experimental prerequisites like steady state conditions, short collecting periods and stable cardiovascular parameters, the choice of the marker for GFR measurements is of utmost importance. The so-called „gold standard" is represented by the determination of inulin clearance (Hagemann and Wüstenberg 1987). Compared to the determination of endogenous creatinine clearance as a measure of GFR, the clearance of inulin is much more reliable (Palnaes-Hansen *et al.* 1997). Basic GFR data reported in the literature depend on the experimental conditions and the method employed. It is therefore difficult to compare the results of different authors without reservation. From this point of view, an attempt was made in the present paper to summarize the bulk of GFR measurements obtained with the same experimental design for better understanding of various factors influencing glomerular filtration capacity in rats. The following aspects of variations in glomerular filtration have been considered:

- time course of GFR in anesthetized rats,
- age-dependent differences of GFR during postnatal development,
- influence of surgical interventions influencing the excretory capacity of kidneys and the liver,
- effects of acute and subchronic drug treatment,
- effects of nephrotoxins.

Method

Animals

The experiments were carried out on female Wistar rats (Han:WIST) of our own outbreed stock. Young animals were nursed by their dams. Adult rats were fed a standard diet (Altromin 1316) and tap water *ad libitum*. Animals were housed under standardized conditions in plastic cages, light-dark cycle 12/12 h, temperature 22±2 °C, humidity 50±10 %.

Experimental design

The rats were anesthetized im. with ketamine (Ursotamin® Serumwerk Bernburg, F.R.G., 7.5 mg/100 g b.w.) and xylazine (Ursonarkon® Serumwerk Bernburg, F.R.G., 1.2 mg/100 g b.w.). The animals received an infusion *via* the tail vein (adult rats) or *via* the jugular vein (developing rats) of isotonic saline containing 4 g/l FITC-inulin at a rate of 4 ml/100 g b.w. per hour. Urine was collected at 30-min periods for 1-3(6) h using an urethral catheter (adult rats) or by transabdominal catheterization of the urinary bladder (developing rats). Blood was collected from the retrobulbar plexus in the middle of each period and at the end of the experiment.

Influence of subchronic drug pretreatment

Triiodothyronine (T_3; SIGMA, St. Louis, U.S.A.) was administered ip. in doses of 20 µg/100 g b.w. once daily for 3 days. *Dexamethasone* (Fortecortin® Mono, E. Merck, Darmstadt, F.R.G.): 60 µg/100 g b.w. were given ip. for 3 days, once daily. Both substances were dissolved in normal saline (1 ml/100 g b.w.). *Epidermal growth factor* (EGF): Animals were pretreated with EGF ([Des-Leu26/Cys(Acm)20/31)EGF (20-31)]; BACHEM Bubendorf, Switzerland) in a dose of 8 µg/100 g b.w. sc. for 8 days, twice daily at 08:00 h and 16:00 h. EGF was dissolved in distilled water (1 ml/100 g b.w.).

Pretreatment with cyclosporine A(CsA): CsA was administered as solution of Sandimmun Optoral ® (Sandoz, Switzerland) orally (2 x 3 mg/100 g b.w., dissolved in 50 µl corn oil). *Diltiazem* (Dilzem®, Gödecke, F.R.G.; 2 x 3 mg/100 g b.w.) was given in 0.5 ml 0.9 % NaCl/100 g b.w. i.p. The controls received the respective solvents only. Pretreatment was made for 19 days at 08:00 h and 20:00 h.

Acute influence of drugs

Amino acid load: Rats were loaded with alanine or arginine (each 20 mg/100 g b.w. x hour) or glutamine (45 mg/100 g b.w. x hour). The amino acids were administered as a continuous infusion together with inulin in normal saline (4 ml/100 g b.w. x hour). In further experiments, glutamine was also administered as a bolus injection (45 mg/100 g b.w.). The injection solutions of alanine, arginine, and glutamine had osmolarities of 380, 365, and 400 mosmol/l, respectively, and pH-values of 6.08, 10.43, and 5.65, respectively. The amino acids were of analytical grade (Sigma-Aldrich, Deisenhofen, F.R.G.).

Influence of a single administration of drugs:

- Furosemide: In adult anesthetized (ketamine/xylazine) rats 0.6 mg/100 g b.w. furosemide (Sigma-Aldrich, Deisenhofen, F.R.G.) were administered intravenously. Inulin was continuously infused as described. In neonatal rats 2.5 mg/100 g b.w. were given intraperitoneally. For the experiments on fetal rats the dams were anesthetized with nembutal. The injection of the test substances (furosemide, inulin) was carried out through the wall of the uterus into the peritoneal cavity of the fetus. The dosage of furosemide and inulin was calculated in these experiments in relation to the mean body mass of the fetus. Fetal and neonatal rats received 2.5 ml isotonic saline solution per 100 g b.w. containing 100 mg inulin and furosemide (Fleck *et al.* 1983).
- p-Aminohippurate (PAH; E. Merck, Darmstadt, F.R.G.): The influence of iv. 200 mg PAH/100 g b.w., dissolved in distilled water (2 ml/100 g b.w.) was measured.
- Tiracizine (Bonnecor®, Arzneimittelwerk Dresden, F.R.G.), an antiarrhythmic drug, was administered ip. in a dose of 0.45 mg/100 g b.w.
- Imidazole: (Sigma-Aldrich, Deisenhofen, F.R.G.) 2 mg/100 g b.w. dissolved in 0.3 ml TRIS buffer, were administered intravenously.
- Cyclosporine A (see above) was administered orally (6 mg/100 g b.w., dissolved in 50 µl corn oil).
- Diltiazem (6 mg/100 g b.w.) was given in 0.5 ml 0.9% NaCl/100 g b.w. ip. The controls received the respective solvents only.

Surgical interventions

Subtotal nephrectomy: 5/6 nephrectomy (5/6 NX) was performed in a two-step surgical procedure under hexobarbitone anesthesia (10 mg/100 g b.w.). At first, the cortex of the left kidney was nearly completely ablated. Three days later the right kidney was removed. Clearance experiments were done six days after the first operation.

Unilateral ligation of the renal artery (20 min ischemia) and contralateral (= unilateral) nephrectomy (UNX) after 24 h: Under hexobarbitone anesthesia, the left kidney was laid bare and the renal artery was ligated for 20 min; for the sham operation, the kidney was freed only without ligating the renal artery. One day later, the right kidney was removed (UNX) to exclude compensatory effects of the contralateral kidney. In sham operated rats, UNX was also performed. Day one of the experiments was the first day after UNX.

Bile duct ligation (DL): The bile duct was ligated in its upper third under hexobarbitone anesthesia. Clearance experiments were done seven days after DL.

Sham operation: Control rats were sham operated: narcosis and opening of the abdominal cavity were without effect on renal functions (for details see Fleck and Bräunlich 1987).

Effects of nephrotoxins

1. Cisplatin: cis-diaminodichloroplatinum(II) (Jenapharm GmbH, Jena, F.R.G. 0.6 mg/100 g b.w.) were given ip. dissolved in 5 ml 0.9% NaCl/100 g b.w.
2. Sodium dichromate (chromate): $Na_2Cr_2O_7$ x $2\ H_2O$ (Serva, Heidelberg, F.R.G.) 1 mg/100g b.w. was administered sc. dissolved in 1 ml 0.9 % NaCl /100 g b.w.
3. Thallium sulphate (thallium): Tl_2SO_4 (Aldrich-Chemie, Steinheim, F.R.G.): 2 mg/100g b.w. were administered ip. dissolved in 5 ml 0.9 % NaCl/100g b.w.

GFR in humans

Eight healthy persons were examined in the Clinic of Internal Medicine of the University of Jena.

Determination methods

1. Fluorescein isothiocyanate (FITC)-inulin (Bioflor, Uppsala, Sweden) was determined spectrofluorometrically (Sohtell *et al.* 1983) in blood and urine samples. Fluorescence was measured at 480 nm excitation and 520 nm emission wavelengths in a HITACHI F-2000 spectrofluorometer.
2. Inulin determination with the anthrone method: In accordance with Führ *et al.* (1955) anthrone (Sigma-Aldrich, Deisenhofen, F.R.G.) was added to urine and serum samples, and after incubation (55 °C, 10 min) colorimetrical measurements were carried out at λ = 578 nm.
3. Enzymatic determination of inulin: In the experiments on human beings inulin was measured enzymatically with inulinase (Boehringer Mannheim, Germany) in accordance with Schmidt *et al.* (1990).
4. Determination of ^{14}C-inulin (NEN, Boston, U.S.A.) was performed with a liquid scintillation counter 81000 (LKB, Sweden).
5. Creatinine determination was done after coupling with picric acid in accordance with the method of Jaffé (Lustgarten and Wenk 1972).

Mode of presentation and statistics

The results are presented as means ± S.E.M. with n=6-9 in each group. The level of significance for

differences between the observations was assessed with the Mann-Whitney-Wilcoxon-test and Student's t-test and considered significant when $p \leq 0.05$.

Results and Discussion

Methodological aspects of the determination of GFR

Compared with other methods (anthrone essay for inulin, ^{14}C-inulin counting, measurement of the clearance of creatinine), the FITC inulin values are in the same range (Table 1). For GFR measurements it is necessary to keep blood inulin concentrations constant. Therefore, in small laboratory animals the clearance studies should be performed under anesthesia and continuous inulin infusion (Ido *et al.* 1992). On the basis of approximately 1000 measurements in anesthetized adult rats of our strain, the mean GFR amounts to 0.86±0.06 ml/[min x 100 g b.w.]. During long-lasting clearance experiments, the GFR is quite stable for 3 h (Fig. 1) and even for 6 h (not shown, see Fleck *et al.* 1989), if the following experimental rules are respected: stable experimental conditions (depth of narcosis, constant cardiovascular status, normal respiration, sufficient hydration of the animals);

- short clearance periods (in adult rats 20-30 min, better 10-15 min);
- steady state inulin plasma concentrations.

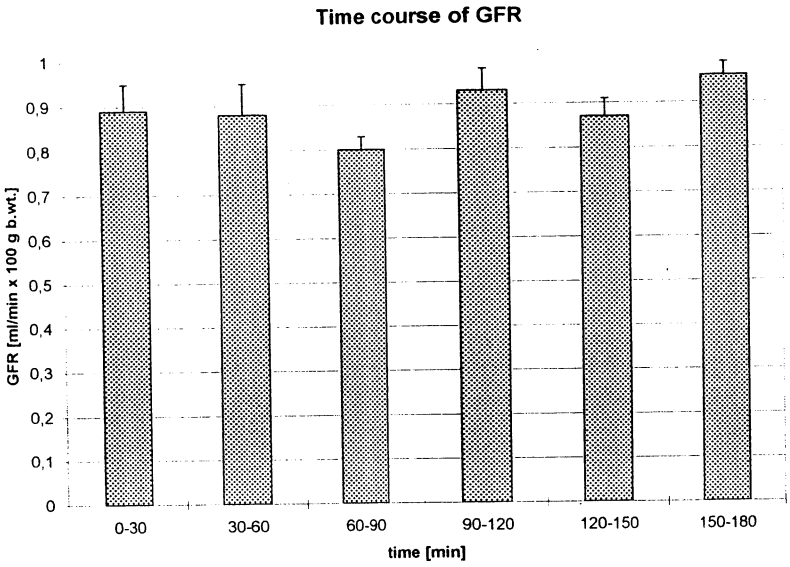

Fig. 1. *Time course of GFR in adult rats during a 3-hour collecting period.*

Table 1. Comparison of different methods for the determination of the GFR in anesthetized (ketamine) adult rats under continuous infusion of 4 ml/100g b.w. 0.9 % NaCl solution.

	Methods employed			
	^{14}C inulin	FITC	anthrone	creatinine
Number of rats	12	12	11	12
Urine volume [µl/(min x 100 g b.w.)]				
Arithmetic mean	37	40	43	41
Standard deviation	11	13	12	16
Variation coefficient [%]	8.96	9.80	8.82	11.77
GFR [ml/(min x 100 g b.w.)]				
Arithmetic mean	0.84	0.86	0.94	0.76
Standard deviation	0.13	0.19	0.36	0.23
Variation coefficient [%]	3.58	6.66	11.51	9.12

(FITC - fluorisothiocyanate-inulin)

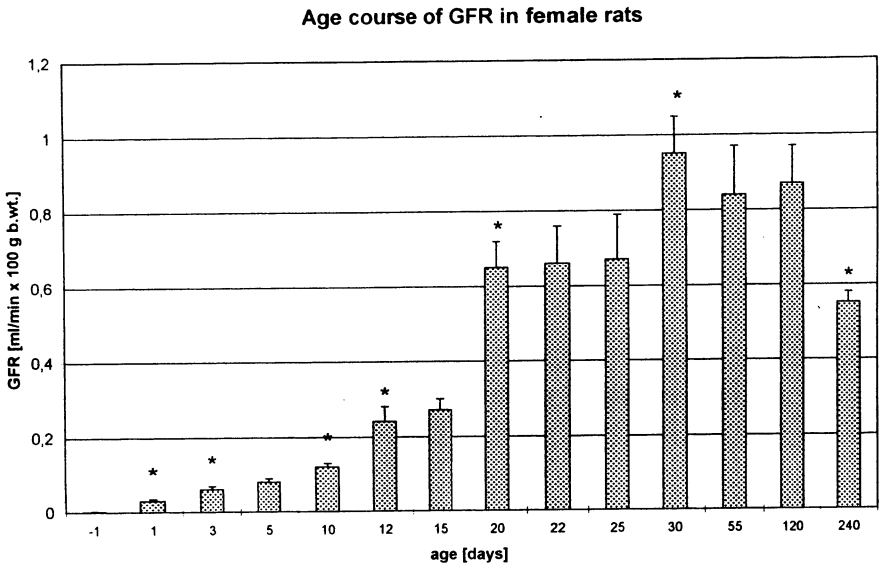

Fig. 2. *Postnatal development of GFR in female Wistar rats. n=6. * significant differences between two subsequent age groups (p<0.05). day – 1 = one day before delivery.*

Developmental changes of GFR

As is known for a number of organ functions (Ankermann *et al.* 1974), GFR develops during postnatal life (Fig. 2). A similar developmental pattern could be shown for other species such as the rabbit (Gouyon *et al.* 1987) or guinea pig (Johnson and Spitzer 1986). The most impressive increase occurs immediately after birth, nevertheless, GFR can also be measured in fetal rats (Fig. 3). Up to the age of one month GFR increases steadily, remains relatively constant up to 4 months and drops significantly thereafter. The maximal filtration capacity is attained by the 30th day of life. This is in good accordance with most organ functions as described e.g. for the metabolic capacity of the liver (Klinger 1996).

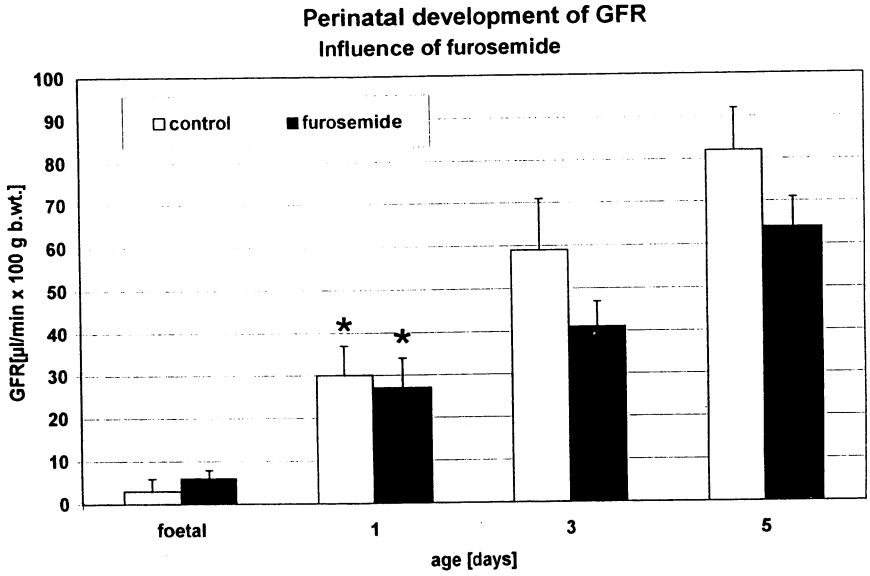

Fig. 3. *GFR of fetal and neonatal rats after administration of furosemide (2.5 mg/100 g b.w.). * significant differences between fetal and neonatal animals (p<0.05).*

The GFR for fetal and neonatal rats is given in Figure 3. Immediately after birth, both GFR and urine production increased significantly (urine flow: 22nd day of gestation: 0.08 ml/[100 g b.w. x hour], 1st day of life: 0.27 ml/[100 g b.w. x hour]). The diuretic action of furosemide can already be demonstrated on 22nd day of gestation: urine flow increased up to 0.22 ml/[100 g b.w. x hour]. In fetal rats, furosemide is without effect on GFR, whereas in neonatal animals GFR is reduced after this diuretic and this decrease is even more pronounced in adult rats (see Fig. 9). This age-related increasing difference of GFR between controls and furosemide-treated rats indicates the so-called phenomenon of compensation after high doses of furosemide. GFR

decreases to compensate the loss of body fluids due to the enhanced urine flow after furosemide. This tubulo-glomerular feedback (Kurokawa 1998) is already effective in the first days of life.

Surgical interventions affecting GFR

As is shown in Figure 4, the removal of kidney tissue is followed by a distinct reduction in kidney functions. The degree of GFR reduction correlates with the amount of renal tissue removed. GFR and urine flow decreased significantly, and blood urea nitrogen was enhanced as a sign of uremia (for details see Fleck *et al.* 1999). Furthermore, uremia in 5/6 NX rats caused a marked inhibition of normal body weight gain, whereas the wet weight of the remaining kidney tissue increased to about 42 % of the weight of two kidneys within six days after ablation of the renal cortex, despite the fact that 83 % of renal tissue had been removed (= 5/6). On the other hand, the compensatory increase in GFR six days after 5/6 NX reached about one third of the controls. This means that, in relation to kidney mass (= 42 % of controls) the compensation of GFR reached only about 80 %, i.e. the same extent of compensation as was found five days after unilateral nephrectomy (see Fig. 5 and the controls in Fig. 6). As expected, the ablation of 50 % of kidney mass reduced GFR by half immediately after surgery (Fleck and Bräunlich 1981). However, already one day later, GFR significantly increased and five days after UNX no longer significantly differed from the controls. As was mentioned above, it only reached about 80 % of the values of rats with two intact kidneys (Fleck and Bräunlich 1984).

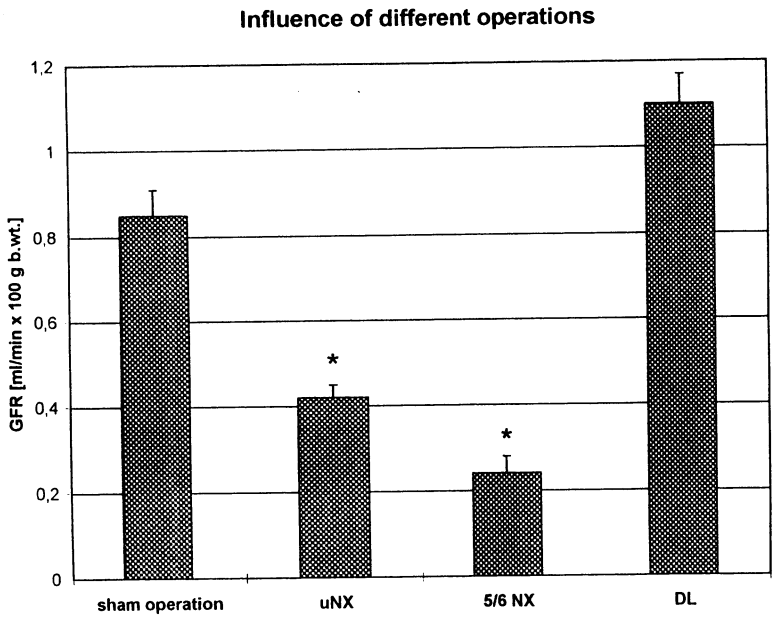

Fig. 4. *Influence of different surgery reducing excretory capacity on GFR in adult rats. UNX = unilateral nephrectomy, NX = bilateral nephrectomy, DL = bile duct ligation. * significant differences compared to sham operation ($p<0.05$).*

Quite comparable results were obtained after renal ischemization (Fleck *et al.* 1993). In contrast to UNX or 5/6 NX, the largest decrease of GFR occurred two days after ischemia (Fig. 6). At this time GFR amounts to about 10 % of controls. Five days after renal ischemia GFR is no longer significantly different from unilaterally nephrectomized control rats. Nevertheless, the compensation of glomerular function after ischemia reaches maximally 80 % of the controls with two kidneys. The reason for this incomplete compensation still remains open. Evidently 80 % of the baseline value seem to be sufficient for survival. It was shown in previous experiments that rats survive 5/6 NX for more than 3 months with slightly enhanced blood urea levels, but without any sign of systemic injury (Bräunlich *et al.* 1986b).

In further studies the relationship between renal and hepatic excretion of drugs was investigated (Fleck and Bräunlich 1991). For this purpose, the biliary excretion was interrupted to characterize the consequences of hepatic failure on renal functions. One week after bile duct ligation (DL, see Fig. 4), a model of the so-called hepato-renal syndrome (Epstein 1994), GFR was slightly, but not significantly enhanced. This means that the occlusion of the biliary excretion pathway is well tolerated in rats. Nevertheless, urine volume was significantly lower in DL rats during 3-hour clearance experiments (not shown). However, urine flow was the

same in DL-rats and controls only immediately after NaCl bolus injection at the beginning of the experiment. The diuretic action of the osmotically active amino acid load (Tietze *et al.* 1992) was evident in both the control (see glutamine, Fig. 9) and DL rats.

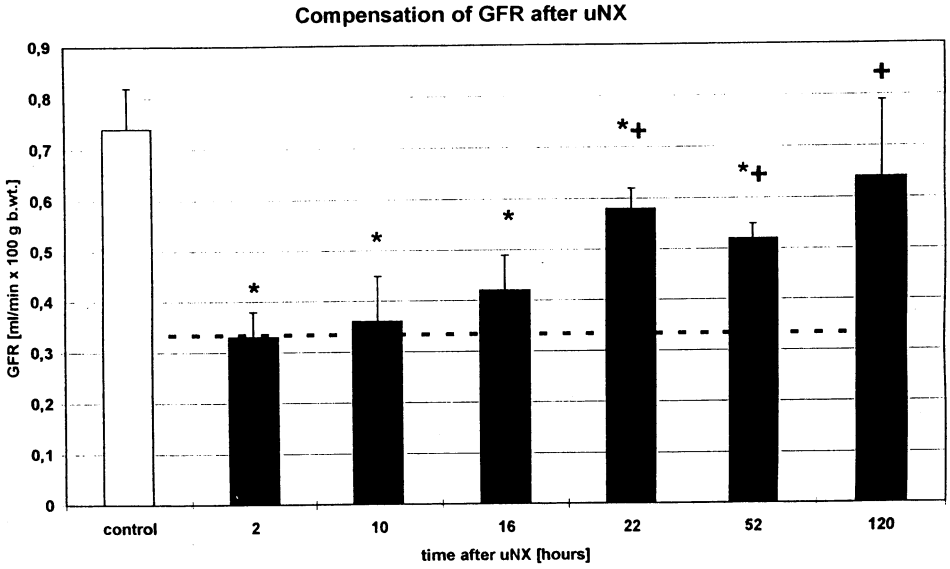

Fig. 5. *Compensation of GFR after unilateral nephrectomy (UNX) in adult rats. Broken line: 50 % of controls (= one kidney). * significant differences between UNX and controls ($p<0.05$). + significant differences between UNX and 50 % of controls ($p<0.05$).*

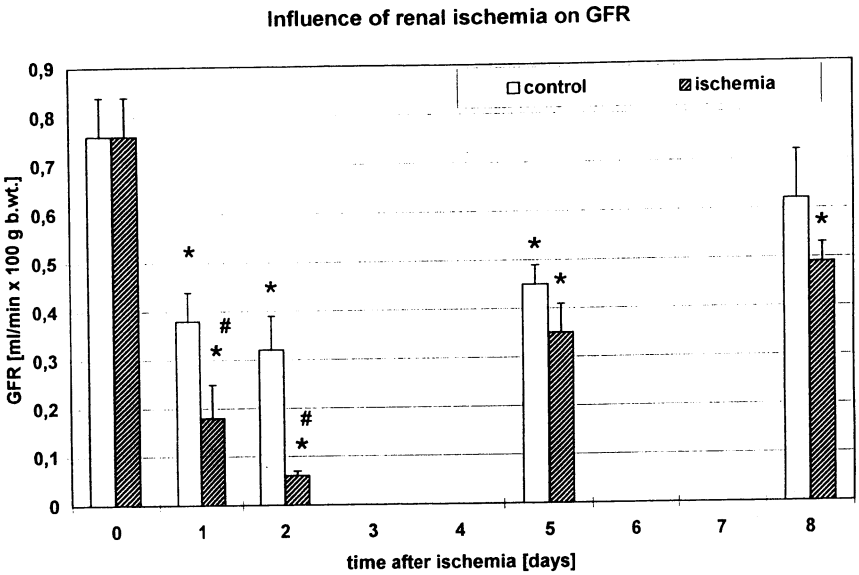

Fig. 6. *Recovery of GFR after 20-min unilateral renal ischemia followed by unilateral nephrectomy (UNX) 24 h later in adult rats. Controls = UNX alone. * significant differences compared to controls before ischemia ($p<0.05$). # significant differences between ischemia plus UNX and UNX alone ($p<0.05$).*

Influence of subchronic drug pretreatment on GFR

A variety of hormonal and vasoactive substances influence glomerular ultrafiltration (Maddox and Brenner 1996), tubular secretion and tubular reabsorption (Fleck and Bräunlich 1995). Especially vasoactive substances such as the antidiuretic hormone, angiotensin II, norepinephrine (for review see Maddox and Brenner 1996), endothelin (Badr *et al.* 1989), leucotriens (Yared *et al.* 1991), platelet-activating factor (Schlondorff *et al.* 1986), and growth factors (Harris *et al.* 1988) can diminish GFR. Interestingly, most vasodilators, e.g. the endothelium-derived relaxing factor (Baylis *et al.* 1992), prostaglandins and acetylcholine (Baylis *et al.* 1976), or histamine (Banks *et al.* 1978) are without effect on GFR.

It was previously shown that renal transport capacity can be stimulated. Both tubular secretion of organic anions (Bräunlich *et al.* 1986a) and tubular reabsorption of amino acids (Fleck *et al.* 1997) can be increased by pretreatment with e.g. dexamethasone, triiodothyronine (T3), or the epidermal growth factor (Fleck and Pertsch 1998). The question arose whether or not the stimulation of renal transport is connected with changes in GFR. It has been found that neither dexamethasone nor T3 influences the GFR after 3 days pretreatment of controls with intact kidneys (Fig. 7). This is in contrast to the findings of Baylis and Brenner (1978) who reported that chronic administration of glucocorticoid hormones increases GFR and renal plasma flow, but the effect of T3 on GFR is not mentioned in the literature at all. On the other hand, GFR is slightly but non-significantly enhanced after both dexamethasone and T3 in 5/6 NX rats. After DL, which is normally followed by a slight increase in GFR (see Fig. 4), dexamethasone normalizes the enhanced GFR, whereas T3 has no beneficial effect on the filtration. On the other hand, subchronic treatment with EGF reduces GFR in rats (Fig. 8). This finding is in good accordance with the results of Harris *et al.* (1988) who reported a decrease in GFR after EGF and those of Keiser and Ryan (1996) describing hemodynamic effects of EGF in rats. The reason for the decrease in GFR consists of a stimulation of prostaglandin production by EGF followed by a constriction of vascular smooth muscles in the kidney (Fisher *et al.* 1989). Interestingly, in rats with an infusion of amino acids, the GFR of EGF-pretreated animals is not significantly different from controls without EGF treatment. Evidently the tubulo-glomerular feedback (Vallon *et al.* 1998) responds to amino acid infusion and is responsible for the increase of GFR in EGF-treated rats. However, GFR is not affected by amino acid *infusion* in controls whereas it was raised significantly immediately after amino acid *bolus injection* (see glutamine, Fig. 9).

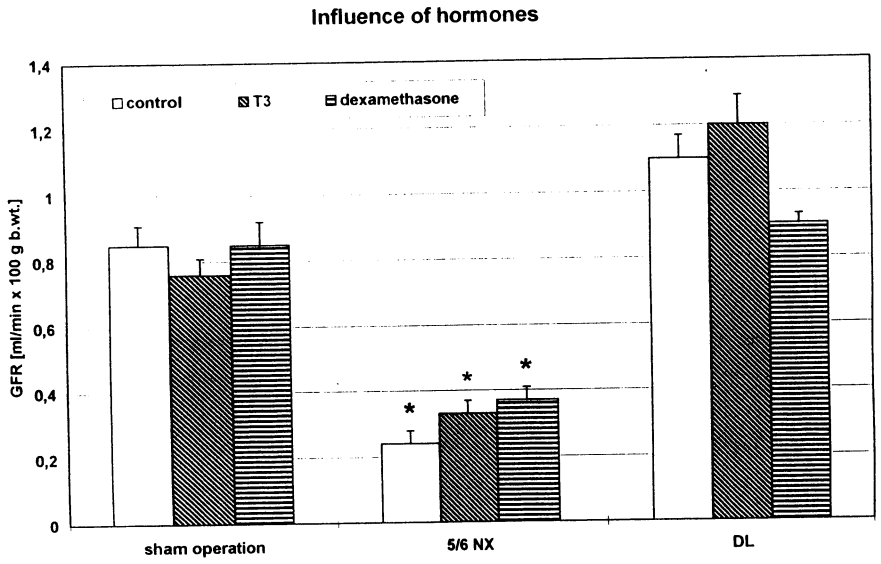

Fig. 7. *Influence of dexamethasone (60 µg/day x 100 g b.w.) and triiodothyronine (T3, 20 µg/day x 100 g b.w.), each hormone given for 3 days, on GFR of sham-operated, 5/6 nephrectomized (5/6 NX) or bile duct ligated (DL) rats. * significant differences compared to corresponding sham-operated group ($p<0.05$). # significant differences between hormone-treated and non-treated group ($p<0.05$).*

Acute influence of drugs on GFR

Immediately after amino acid bolus injection, the osmotically active amino acids caused an increase in urine flow (not shown), probably also *via* a tubulo-glomerular feedback, GFR increases. This was the only case when a substance had an acute enhancing effect on GFR in our studies. Other compounds and drugs, the effect of which was tested acutely, had either no effect on GFR (PAH, tiracizine, diltiazem) or they reduced GFR significantly. This reduction is due to a tubulo-glomerular feedback as mentioned for furosemide and amino acid infusion or it is a symptom of nephrotoxicity as has been reported for heavy metals (Fleck and Appenroth 1996) and cyclosporine A (Kuhn *et al.* 1998).

Effects of nephrotoxins on GFR

However, the reason for diminished in GFR is different in the case of heavy metals and CsA: After administration of chromate (Bräunlich *et al.* 1992), cis-platinum (Appenroth *et al.* 1997) or thallium (Fleck and Appenroth 1996) morphological changes have been described in the glomeruli. Nevertheless, the reduced GFR after CsA seems to be due to disturbances in renal blood flow (Cavarape *et al.* 1998). The opposite is true

for the effect of imidazole on GFR. In this case a diminution of renal blood flow (via prostaglandin synthesis inhibition) does not occur (Balint and Laszlo 1985) and can not hence be responsible for the reduction in GFR.

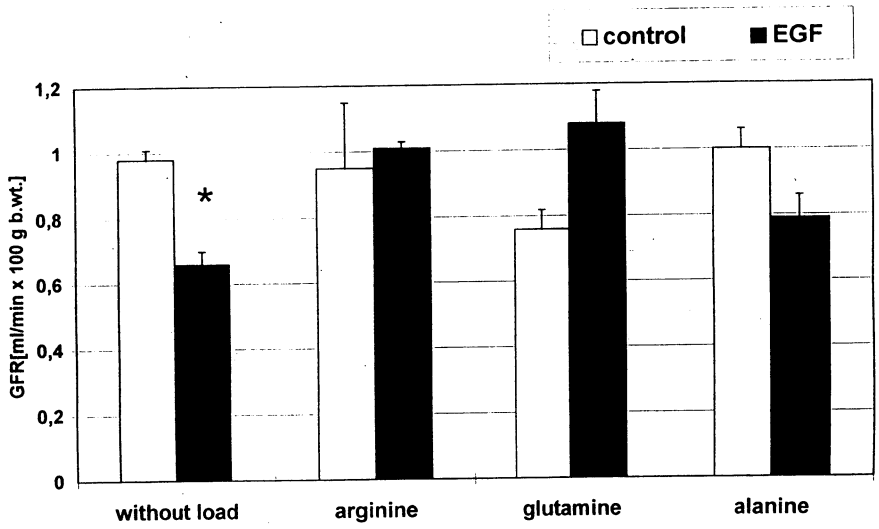

Fig. 8. *Effect of epidermal growth factor (EGF, 8 µg/100 g b.w. for 8 days, twice daily) on GFR in amino acid-loaded rats. * significant differences to controls ($p<0.05$).*

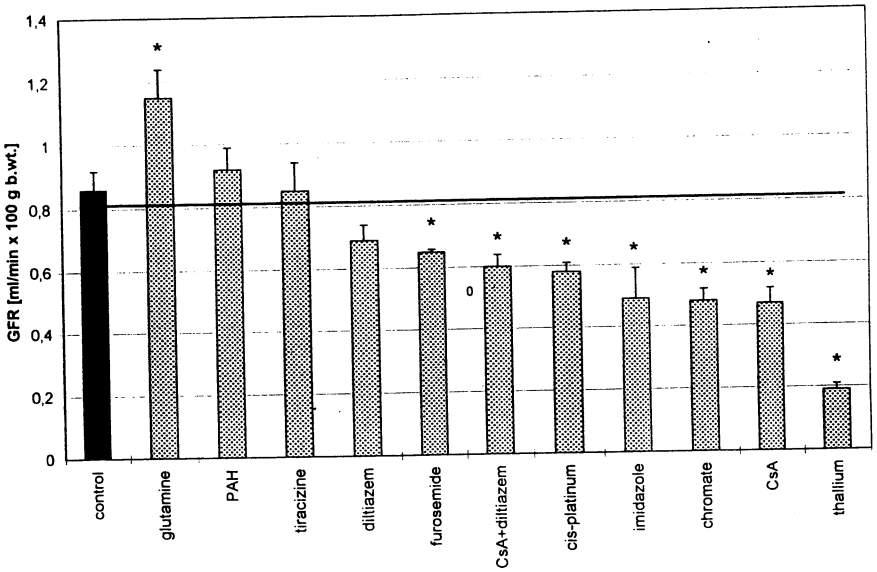

Fig. 9. *Influence of various drugs or nephrotoxins on GFR in adult rats. PAH: p-aminohippurate, CsA: cyclosporine A. * significant differences compared to controls (= continuous line) ($p<0.05$).*

In further experiments we tested whether or not the nephrotoxic effect of CsA can be prevented by concomitant treatment with diltiazem. This combination has been reported to be beneficial in kidney transplanted patients (Sperschneider *et al.* 1997). Unfortunately, in our experiments on rats no beneficial diltiazem effect at all could be found. After long-lasting pretreatment with diltiazem its GFR depressing effect (see Fig. 9) disappeared, but diltiazem did not prevent the reduction of GFR under CsA therapy (Fig. 10). Some data such as morphological findings (unpublished data), metabolic interference between CsA and diltiazem (Kuhn *et al.* 1996) and species differences between patients and rats indicate that the rat is not suitable for CsA nephrotoxicity studies.

Species differences

On the basis of these species differences, we have compared the GFR values in rats and our patients with literary data for various species. It is known that GFR is in general inversely correlated with body mass or body surface (Donadio *et al.* 1997). This means that the GFR in the mouse (Field *et al.* 1991) and rat is the highest, whereas that of human beings is relatively low, comparable to those of newborn rats and rabbits (Gouyon *et al.* 1987).

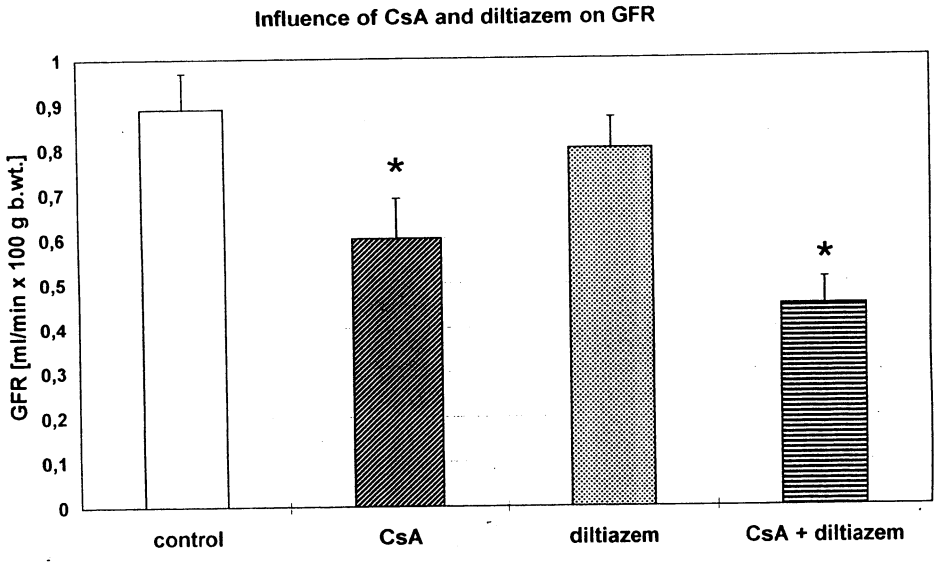

Fig. 10. *Lack of beneficial effect of diltiazem on GFR in cyclosporine A (CsA) nephrotoxicity in adult rats. * significant differences compared to controls ($p<0.05$).*

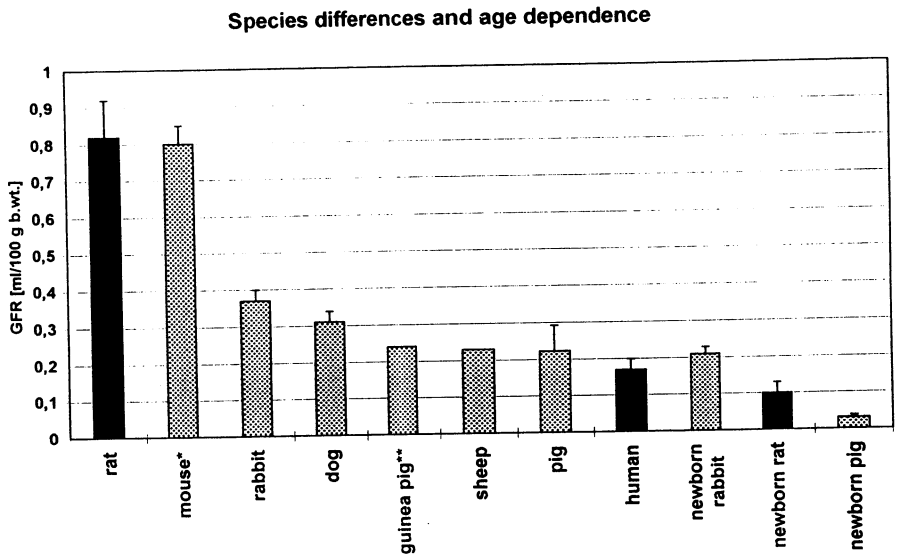

Fig. 11. *Species differences and age dependence of GFR. For references see: Kaufman and Bergman (1978), Link et al. (1985), Johnson and Spitzer (1986), Gouyon et al. (1987), Fine (1988), Field et al. (1991), Lortholary et al. (1993 Bauer et al. (in preparation); black columns = own results; n=6. * per g kidney weight. ** isolated perfused kidney.*

Conclusions

The measurement of the FITC inulin clearance can be recommended as the method of choice for the determination of GFR in small laboratory animals. This technique is both easy to perform and very well reproducible. Despite the ability of the kidney to maintain constant RBF and GFR over a wide range of perfusion pressures, many factors influencing glomerular filtration have to be considered. The following factors which may interfere with GFR are of practical importance:

- Physiological development of GFR during postnatal development
- Species and sex differences
- Reduction in renal mass

- Renal ischemia
- Compensatory capacity after renal or hepatic failure
- Hormone treatment
- Diuretics
- Hyperfiltration after administration of osmotically active substances
- Reduction of GFR caused by:
  - Negative tubulo-glomerular feedback
  - Decreased renal perfusion
  - Morphological destruction of glomerular
- integrity by nephrotoxins.

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