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A. Verbaeys · S. Ringoir · G. Van Maele · N. Lameire

Influence of feeding, blood sampling method and type of anaesthesia on renal function parameters in the normal laboratory rat

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Abstract With sophisticated experiments it is necessary to handle laboratory animals many times. To determine the effect of minor handling a series of experiments was performed to measure the impact of fasting, anaesthesia, blood collection method and serum creatinine analysis on renal function. Simple clinical methods to measure renal function parameters such as diuresis, urinary osmolality, urinary creatinine excretion and serum creatinine were used. During fasting a significant increase ($P< 0.01$) in diuresis and a significant decrease ($P< 0.01$) in urinary osmolality were noted. Fasting and anaesthesia have the additional effect of significantly decreasing ($P< 0.05$) urinary creatinine excretion. Blood sampling method also has a significant impact on serum creatinine: venous sampling causes false-positive differences compared with simultaneous arterial sampling.

Key words Serum creatinin · Normal laboratory rat
Renal function parameters · Blood sampling

Assessment of renal function parameters in laboratory animals such as the rat is frequently necessary in many experimental settings. For example, many studies on the nephrotoxicity of investigative or therapeutic drugs involve the follow-up of relatively simple biological renal function tests, such as those on daily diuresis, urinary osmolality, urinary creatinine excretion and serum creatinine levels.

As far as we know, early scientific tables did not mention normal values for serum creatinine and other renal function parameters of laboratory animals [2, 10], while more recent work shows that some investigational manipulations may influence renal function [11].

Feeding status, handling of the animals, blood sampling technique and methodology of analysis, type of anaesthesia and statistical methodology may all influence the results. The present paper describes the influence of several of these variables on the development of these parameters in the normal laboratory rat.

A. Verbaeys (✉)
Department of Urology, University Hospital Gent, De Pintelaan 185, B-9000 Gent, Belgium, Fax: +33 (91) 2403889

S. Ringoir · N. Lameire
Department of Nephrology, University Hospital Gent, De Pintelaan 185, B-9000 Gent, Belgium

G. Van Maele
Department of Medical Information, University Hospital Gent, De Pintelaan 185, B-9000 Gent, Belgium

Methods

All animals studied were male Sprague-Dawley rats 3 months old and weighing approximately 300 g. In all cases where urine sampling was necessary, the animals were kept in metabolic cages to allow complete collection of the urine under paraffin to prevent evaporation. In order to allow the animals to become acquainted with their environment, all experiments were started 48 h after the rats were moved to their new housing. All animals were fed normal rat chow (Table 1) and received tap water ad libitum, unless mentioned otherwise. Four different protocols were followed, which are represented graphically in Fig. 1. All the experiments were performed in accordance with the Helsinki Declaration for Animal Research.

First protocol: definition of normal values

Experiments were performed to establish the normal values for diuresis, urinary osmolality, creatinine excretion and serum creatinine in 22 unmanipulated rats, residing in metabolic cages. After the acquaintance period, urine was collected over 48 h, at the end of which period a blood sample (B48) was taken from the left jugular vein, with the animal under short ether anaesthesia.

Second protocol: influence of fasting without anaesthesia

Experiments were performed to assess the influence of fasting alone on diuresis, urinary osmolality and urine creatinine excretion. Body weight, food consumption and fluid intake of the animals were followed. After the acquaintance period of 48 h, eight animals were followed in metabolic cages for four consecutive periods of 24 h (day 1, day 2, day 3 and day 4). All 24-h urine was collected every day and no blood sampling was performed. No fluid restriction was applied but food was supplied only on the 1st, 3rd and 4th days and withheld during the 2nd day.

Table 1 Normal rat chow: ingredients and guaranteed basic composition

Ingredients	
Naked barley	20.0%
Concentrate	2.0%
Cassava roots	20.0%
Sugarcane molasses	3.0%
Soybean oil	0.5%
Soybean meal 44	0.9%
Soybean whole seeds	5.0%
Soft wheat	10.0%
Wheat feedmeal	15.0%
Sunflowerseed meal 28	15.5%
Guaranteed basic composition	
Crude protein	17%
Fat	4%
Sugar and starch	35%
Cellulose	10%
Ash	7%

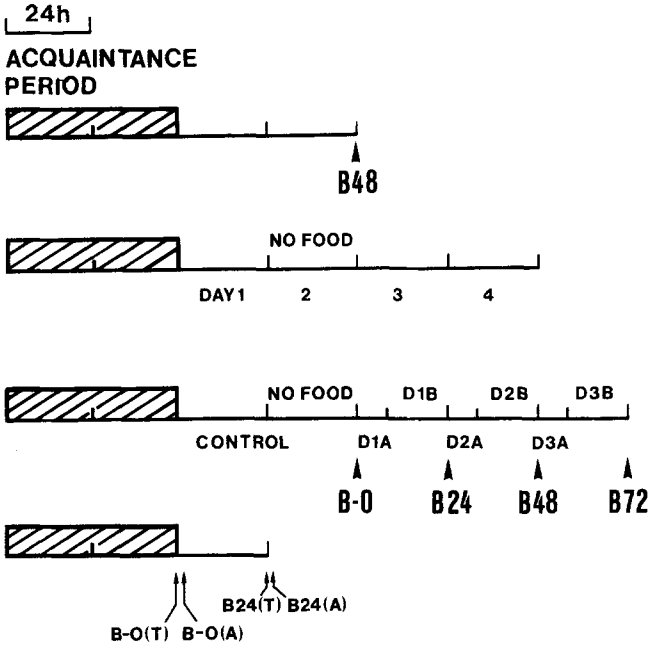

Fig. 1 Schematic representation of all four protocols: *upper graph*, first protocol, *second graph*, second protocol, *third graph*, third protocol, *lower graph*, fourth protocol, *B*, blood sampling, *B(T)*, blood sampling from tail, *B (A)*, blood sampling from artery

Third protocol: influence of fasting and anaesthesia

These experiments were performed in seven rats to assess the influence of a combination of fasting and anaesthesia on some renal functional parameters. After the 48-h acquaintance period, the urine was collected for the next 48 h as a control, during which period free fluid intake was allowed. After 24 h the food was withdrawn, but not the access to water. At the end of this 48-h control period, a blood sample was taken (B-O) with the animal under short ether anaesthesia by amputation of the distal 5 mm of the tail. If necessary, the tail was "milked" in order to obtain a sufficient amount of blood. To prevent subsequent bleeding, the tail end was ligated. After this first anaesthesia, the animals were then followed for a further 3 days. Each 24-h interval was divided into a first period of 8 h (D1A, D2A, D3A) and a second period of 16 h (D1B, D2B and D3B) for day 1 (D-1), day 2 (D-2) and day 3 (D-3), respectively. At the end of each day, blood samples (B24, B48, B72) were obtained by tail amputation with the animal under ether anaesthesia, as described above.

Fourth protocol: influence of blood sampling methods and creatinine analysis

These experiments were designed to explore the influence of different techniques of blood sampling and different methods for blood creatinine analysis.

Eight rats were followed for 24 h after the acquaintance period. Two blood samples (B-O) were taken with the animal under ether anaesthesia at the beginning of the 24-h period: a first sample [B-O(T)] was taken from the tail after amputation of the distal end and a second sample from the carotid artery [B-O(A)] after a minimal blunt dissection of the neck. The carotid artery was ligated after puncture and the small neck skin incision was sutured. At the end of the 24-h period, an additional two blood samples (B24) were taken with the animal under anaesthesia with i.p. pentobarbital (60 mg/kg BW). One blood sample was taken after tail amputation (i.e. the second amputation) [B24(T)] and one after puncture of the abdominal aorta [B24(A)] after a small laparotomy.

All serum creatinine determinations were performed by the Jaffé method, using a Creatinine Analyser II (Beckman Clinical Instruments Division, Fullerton, USA) [3] except for every second creatinine determination in the fourth protocol, which was performed with the enzymatic colorimetric creatinine PAP method [5].

Urinary osmolality was measured with an Advanced Digimatic Osmometer (Model 3DII, Needham Heights, Mass., USA) [7]. The urinary creatinine excretion was measured by the Jaffé reaction. The units used for diuresis were µl/min 100 g BW, for urinary osmolality mosm/kg, for urinary creatinine excretion µg/min 100 g BW and for serum creatinine µmol/l (and mg%). The results are expressed as medians (M) and interquartile ranges (IR), the latter always given in parentheses. To evaluate the results obtained in one group of animals, a Friedman analysis was performed. The Mann-Whitney U-test was used to compare the results obtained between two different groups of animals. A P value of less than 0.05 was considered to be significant. All results are graphically represented by box-and-whisker plots.

Results

First protocol: definition of normal values (Fig. 2)

The median values for diuresis, urinary osmolality, urinary creatinine output and serum creatinine were 2.96 µl/min 100 g BW (IR, 0.95), 2075 mosm/kg (IR, 706), 0.0259 µmol/min 100 g BW (IR, 0.0034) and 51.9 µmol/l (IR, 15.0) [0.59 mg% (IR, 0.17)], respectively.

Second protocol: influence of fasting without anaesthesia (Figs. 3, 4)

Figure 3 shows the evolution of water intake, food consumption and body weight over the 4 experimental days. During fasting, the fluid intake decreased significantly ($P< 0.001$) and a parallel decrease in body weight was observed ($P< 0.05$). On the other hand, food consumption observed the day after the fasting period was higher [food consumption on day 3 significantly higher than on day 4 ($P< 0.05$)].

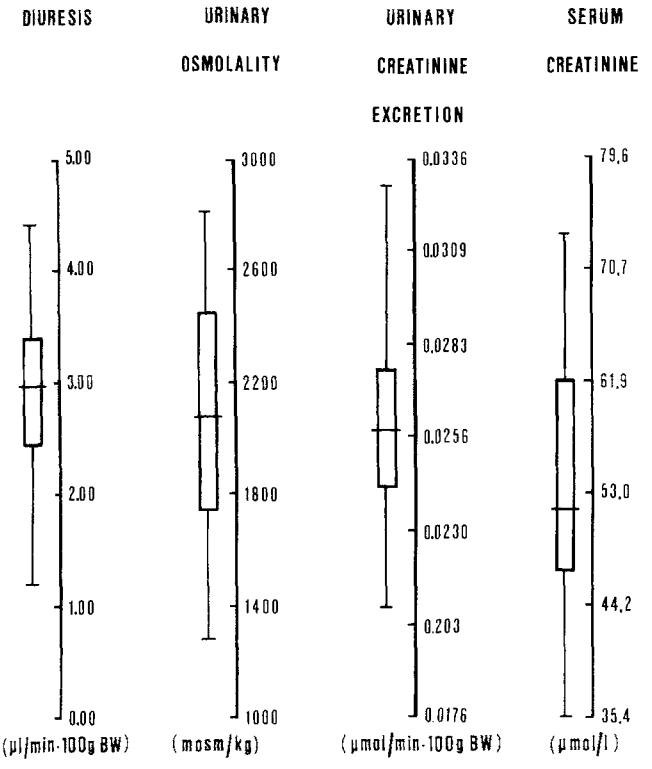

Fig. 2 Box-and-whisker plots for diuresis, urinary osmolality, urinary creatinine excretion and serum creatinine concentration

Figure 4 illustrates the development of diuresis, urinary osmolality and urinary creatinine excretion over a 4-day period. It is clear that during the 24 h of fasting, diuresis was significantly higher (P=0.007) than during the subsequent days. During this 2nd day, the urinary osmolality decreased significantly (P=0.006) compared with all other values. There was a tendency for urinary creatinine excretion to decrease, although the differences were not statistically significant.

Third protocol: influence of fasting and anaesthesia (Fig. 5)

The results and analysis by the Mann-Whitney U-tests used to compare the values of the different parameters obtained during the control period (first 48 h) in protocol 3 with the normal values as obtained in protocol 1 are shown in Table 2. Significantly lower values for urinary osmolality and urinary creatinine excretion and a significant increase in serum creatinine were observed.

Figure 5 illustrates the development of diuresis, urinary osmolality, urinary creatinine excretion and serum creatinine. After each ether anaesthesia, diuresis decreased in the first 8 h but returned to normal values during the second period of every 24-h interval. Only the decrease in diuresis observed after the third narcosis was statistically sig-

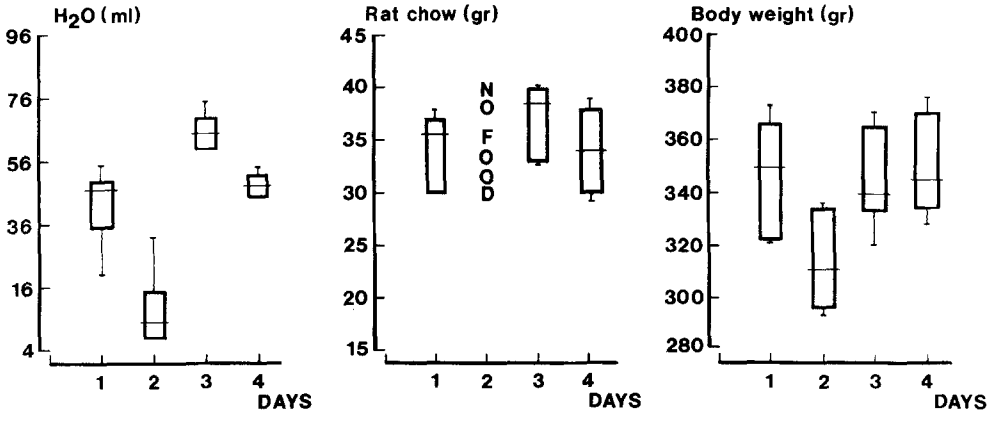

Fig. 3 Evolution of food consumption, fluid intake and body weight. Fluid intake: day 2 value is significantly lower than all other values (P< 0.001) and day 3 value is significantly higher than all other values (P< 0.001). Food consumption: day 3 value is significantly higher than day 4 value (P< 0.01). Body weight: day 2 value is significantly lower than all other values (P< 0.05)

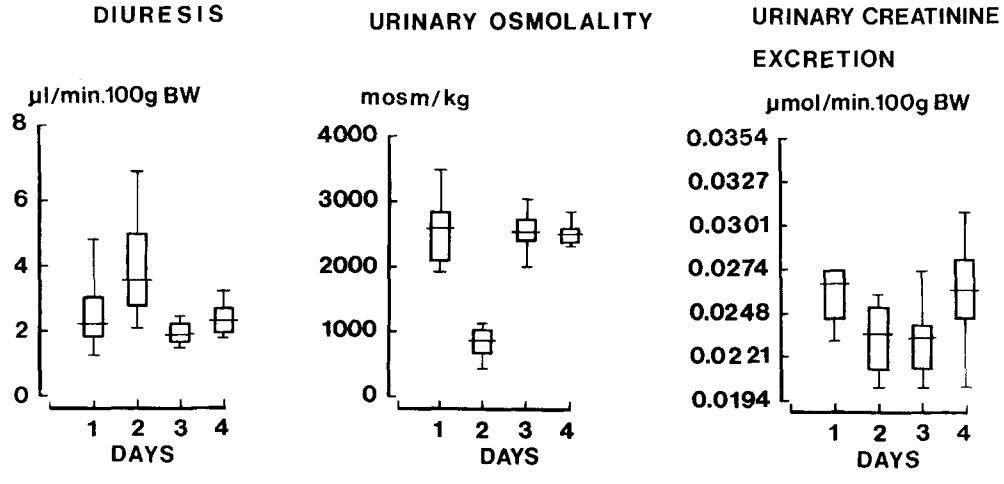

Fig. 4 Influence of fasting on diuresis, urinary osmolality and urinary creatinine excretion. Diuresis: day 2 value significantly higher than day 3 and day 4 values (P< 0.01). Urinary osmolality: day 2 value significantly lower than all other values (P< 0.01). Urinary creatinine excretion: no statistical differences

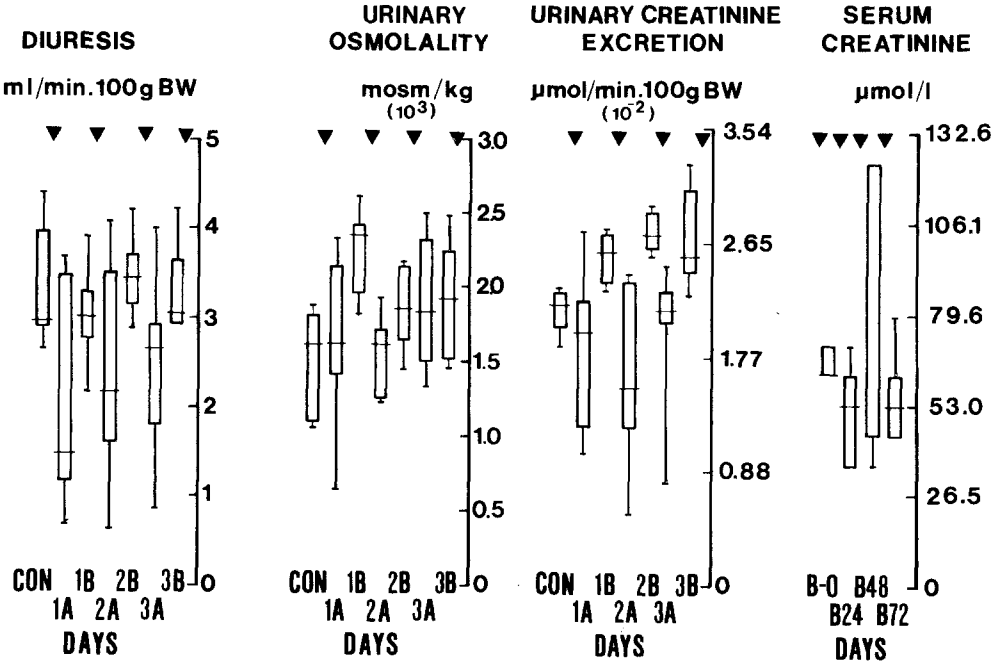

Fig. 5 Influence of fasting and anaesthesia on diuresis, urinary osmolality, urinary creatinine excretion and serum creatinine. Diuresis: D3A significantly lower than D2B ($P< 0.05$). Urinary osmolality: D1B significantly higher than all other values ($P< 0.05$). Urinary creatinine excretion: D1B, D2B and D3B significantly higher than all other values ($P< 0.05$). Serum creatinine: B24 significantly lower than control ($P< 0.05$). ▼ Indicates each narcosis

Table 2 Results of Mann-Whitney U-tests comparing the median of the controls with the median of the normal values (*NS*, not significant)

Parameter	Median (IR) for normal value	Median (IR) for control value	Mann-Whitney U-test
Diuresis (µl/min 100 g BW)	2.96 (0.95)	2.95 (1.06)	NS
Urinary osmolality (mosm/kg)	2075 (706)	1610 (730)	$P< 0.002$
Urinary creatinine excretion (µmol/min 100 g BW)	0.0259 (0.0034)	0.0217 (0.0026)	$P< 0.001$
Serum creatinine (µmol/l)	51.90 (15.0)	61.88 (8.84)	$P< 0.005$
mg%	0.59 (0.17)	0.70 (0.10)	

nificant compared with the value prior to this anaesthesia ($P< 0.05$).

Compared to the first 8-h period, urinary osmolality increased during every second period of the 24-h interval. The value for the D1B period was significantly higher than all other values ($P< 0.05$).

The first 8 h following each narcosis, urinary creatinine excretion decreased but these values normalized over the subsequent 16 h. The values obtained during D1B, D2B and D3B were not only significantly higher than the control values (CON), but were also significantly higher than the value obtained in the preceding period ($P< 0.05$).

Only the B24 value of the serum creatinine was significantly lower than the control value (B-O) ($P< 0.05$).

Fourth protocol: influence of method of blood sampling and serum creatinine analysis (Fig. 6)

When blood was taken from the tail, there was a significant difference between the first sample [B-O(T)] and the second, taken 24 h later [B24(T)], regardless of the method of analysis. When the analyses were performed on arterial blood, no statistically significant differences between B-O(A) and B24(A) were observed. In both tail and arterial blood samples the differences between the Jaffé method and the enzymatic method were highly significant, giving a systematic overestimation of the creatinine concentration by the Jaffé method.

Discussion

The experiments of the first protocol were performed to evaluate what could be considered to be "normal" values for diuresis, urinary osmolality, urinary creatinine excretion and serum creatinine for the animals used in these experiments. Our normal values are in complete agreement with the data kindly provided by the IFFA CREDO animal laboratory for rats of the same age and body weight [8].

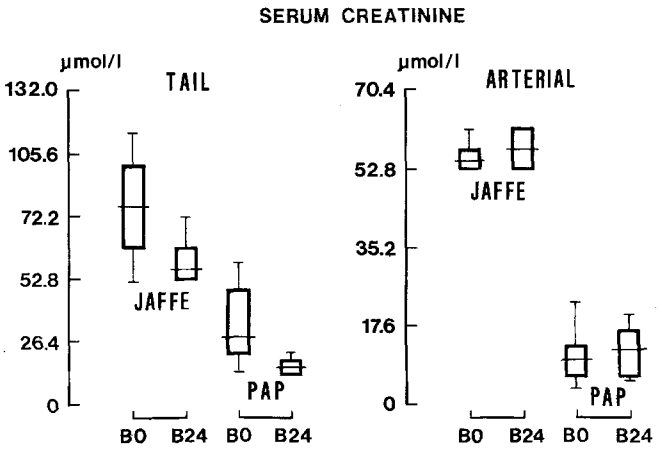

Fig. 6 Tail blood and arterial blood analysed by Jaffé's method and by enzymatic colorimetric PAP method. Tail blood by Jaffé: there is a significant difference between B-O and B24 ($P< 0.05$). Tail blood by PAP: there is a significant difference between both values ($P< 0.05$). Arterial blood by Jaffé: no statistical difference. Arterial blood by PAP: no statistical difference (*B-O*, first blood sample ; *B24*, sample taken 24 h later)

In the second protocol, a significant increase in diuresis and a significant decrease in urinary osmolality were observed during the 2nd day of the experiments, corresponding with the day of fasting.

The increase in diuresis was certainly not due to an increase in water intake, as the latter was significantly lower ($P< 0.01$) during fasting. In parallel with fasting, a lower fluid intake and an increased output of diluted urine was observed. The urinary creatinine output also tended to decrease during fasting but a statistically significant difference was not obtained.

In the third protocol, the control period lasted 48 h. The fact that the animals were not allowed to eat for 24 h prior to the first anaesthesia did not influence diuresis, measured over the 48 h preceding this narcosis. However, after each anaesthesia, a decrease in diuresis was observed in the 8-h period following this narcosis. This decrease was followed by a normalization of the diuresis over the subsequent hours.

The low urinary osmolality in the control period was due to the 24 h of fasting. Indeed, the results obtained during fasting in the second protocol showed a significant decrease in urinary osmolality. Fasting during the 24-h control period was the only difference in the experimental setting between the first and the third protocol. The high D1B urinary osmolality value could be explained by extra food intake of the animal to compensate for the preceding fasting period. This was not yet the case in the first period of the 1st day (D1A) since the animals were not able to compensate with food intake during the recovery of their preceding narcosis.

As expected, fasting also influences the urinary creatinine output (see Mann-Whitney U-test in Table 2). After each period of narcosis, the urinary creatinine excretion was lower than the value obtained during the second period of each day. The fact that animals recovering even from a short ether narcosis eat and drink less might be responsible for the observed fluctuations in the different parameters.

When food and water intake was normal, as it was during the D1B, D2B and D3B periods, the median values of the urinary creatinine output returned to values identical to the normal value.

The control value for the serum creatinine in the third protocol is significantly higher than the normal value (see Mann-Whitney U-test in Table 2). Since we observed that fasting animals in the second protocol also show a significantly lower water intake, this higher serum creatinine value could be explained by dehydration.

The blood sampling technique and the applied analytical method both seem crucial when evaluation of serum creatinine values is concerned. The first blood sampling from the tail in the fourth protocol was often difficult and then "milking" of the tail was necessary to obtain a sufficient amount of blood. The latter maneuver probably causes some muscle damage with release of creatine. As the PAP method is based on the enzymatic conversion of creatinine to creatine by creatininase, a falsely elevated serum creatinine can therefore be obtained. "Milking" the tail may also induce red cell damage with release of haemoglobin in the plasma. As the Jaffé method is based on colorimetric analysis, it is possible that still higher creatinine values are obtained due to the presence of chromogens in the serum. This can explain the differences observed between the Jaffé and the enzymatic method.

The first tail amputation almost certainly caused an inflammatory process. When the second tail amputation was performed, the blood sampling was much easier and additional "milking" was not necessary, explaining the lower values with both analytical methods after 24 h. The B-O values taken from the tail and the carotid artery revealed clear differences, regardless of the method of analysis. The comparison of the B24 samples showed almost identical results with both analytical methods. Some caution in the interpretation of serum creatinine values, obtained by tail amputation, is therefore warranted.

In conclusion, our data clearly illustrate the influence of fasting on some urinary parameters such as osmolality and diuresis in the Sprague-Dawley rat. Furthermore, anaesthesia of the animals clearly influences their urinary creatinine excretion. The blood sampling method and, to a lesser extent, the analytical methodology played a role in the results obtained. Although we did not perform investigations with other rodent species or with rats of the same strain but of a different sex, age and/or weight, we believe that great care should be observed when commenting on results obtained from experiments with laboratory animals subjected to fasting and/or anaesthesia and/or blood sampling. As far as we know, publications concerning these fundamental principles in laboratory research are not available.

We were surprised to note that in many papers the method of blood sampling in the experimental animals is not always described exactly [1, 4, 9]. It is obvious that the

manipulations performed in the present study were relatively minor compared to those required in certain experimental conditions [6, 12]. We believe that our results indicate that whatever experimental manipulations are performed in Sprague-Dawley rats, they can greatly influence the final results.

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