J. Physiol. (1976), **262**, *pp.* 119–129
With 4 text-figures
Printed in Great Britain

FUNCTIONAL AND MORPHOLOGIC MATURATION OF SUPERFICIAL AND JUXTAMEDULLARY NEPHRONS IN THE RAT

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(*Received 23 February 1976*)

SUMMARY

1. The development of single nephron glomerular filtration rate (SNGFR) was studied in both superficial and juxtamedullary nephrons in rats in relation to concomitant morphologic maturation. These experiments were carried out in rats between 23 and 91 days of age (between 36 and 275 g body weight) with the [^{14}C]ferrocyanide infusion technique.

2. SNGFR of the superficial and juxtamedullary nephrons increased with body weight, glomerular volume and proximal tubular length.

3. The ratio SNGFR of the superficial (S) nephrons/SNGFR of the juxtamedullary (JM) nephrons rose from 0·60 in the 40–60 g rats to 0·84 in the adult rats, demonstrating the centrifugal functional maturation of the nephrons.

4. The S/JM ratio for both glomerular volume and tubular length was constant and averaged $0{\cdot}72 \pm 0{\cdot}12$ and $0{\cdot}81 \pm 0{\cdot}05$, respectively, indicating that while the increase in SNGFR was greater for S than for JM nephrons, this was not accompanied by concomitant disproportionate increases of glomerular volume and/or proximal tubular length between these nephron categories during development in the rat.

INTRODUCTION

Morphologically, renal maturation follows a centrifugal pattern, at any stage of post-natal maturation the oldest nephrons are at the corticomedullary junction, and the youngest in the subcapsular area (Fourman & Moffat, 1971; Goncharevskaya & Dlouha, 1975). As far as functional maturation is concerned studies of developmental changes in renal blood flow distribution in canine puppies have shown a disproportionately high increase with time in the superficial cortical component (José, Logan, Slotkoff, Lilienfield, Calcagno & Eisner, 1971; Kleinman & Reuter, 1973;

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Olbing, Blaufox, Aschinberg, Silkalns, Bernstien, Spitzer & Edelmann, 1973). Single nephron glomerular filtration rate (SNGFR) of the superficial nephrons was measured by several investigators in the growing animal. From data collected in the dog, Horster (Horster, Kemler & Valtin, 1971; Horster & Valtin, 1974) calculated that the percentage of measured kidney glomerular filtration rate (GFR) accounted for by the filtration rate of superficial nephrons changed significantly from about 60–85 between the first and fourth month of post-natal development in the dog. This observation points to a functional maturation proceeding from the inner toward the outer cortex. Spitzer & Brandis (1974) observed in the guinea-pig during the first 15 days of life an average rate of increase in glomerular filtration rate per nephron of 0·48 nl./min.day which was more than twice the observed rate of increase in the superficial nephrons (0·21 nl./min.day). Thus the initial increase in total kidney glomerular filtration rate was primarily a consequence of the activity of the deep nephrons. During the next 2 weeks, in contrast the increase in superficial SNGFR (0·97 nl./min. days) was greater than the average increase for all nephrons (0·71 nl./min.day).

The purpose of this study was to examine directly and simultaneously the development of SNGFR as assessed by the [^{14}C]ferrocyanide technique (Rouffignac, Deiss & Bonvalet 1970) in both superficial and juxtamedullary nephrons in rats in relation to concomitant morphologic maturation. The particular value of the data consists in the fact that they have been obtained on both superficial and juxtamedullary nephrons simultaneously.

METHODS

The experiments were carried out on fifteen female Wistar strain rats, weighing from 36 to 275 g, aged between 23 and 91 days. They were normally hydrated but starved for 18 hr immediately before experiments. Anaesthesia was induced by Nembutal I.P. (5 mg/100 g) and maintained by additional intermittent I.V. doses (0·5 mg). The rats were tracheotomized and a catheter was introduced into the right jugular vein. A PE10 catheter was introduced into a femoral artery and threaded up until its end was positioned slightly above the two renal arteries. This catheter was used for the injection of the unlabelled ferrocyanide pulse at the end of the experiments. Another catheter (PE50) was introduced into the contralateral femoral artery and connected to a strain gauge pressure transducer for the continuous monitoring of the arterial blood pressure. Both ureters were cannulated and a loose ligature was placed around the left renal pedicle to be used at the end of the experiment for the quick removal of the kidney, following the administration of the unlabelled ferrocyanide pulse. Once the surgical preparation was completed, an infusion of a 0·9% NaCl solution containing 20 μC/ml. of tritiated inulin was started at a rate of 15 μl./min. 100 g. After a period of 1 hr for equilibration, ureteral urine from both kidneys was collected over 20 min clearance periods. In eleven out of the fifteen rats the single nephron glomerular filtration rate (SNGFR) was then measured with the [^{14}C]ferrocyanide infusion technique (Rouffignac *et al.* 1970). At the end of the experiment, the

right kidney was dissected free of capsular and hilar fat and wet weighed. The number of glomeruli was then determined using the method described by Damadian (Damadian, Shawayri & Bricker, 1965). Morphological data were collected according to Baines & Rouffignac (1969).

Measurement of SNGFR

After a 100 μc/100 g priming dose, ^{14}C-labelled Na ferrocyanide was infused at a rate of 10 μc/min. The Na-ferrocyanide concentration in the infusion was raised to 50 mg/ml. in order to equilibrate the plasma concentration of ferrocyanide above 1 m-mole/l. Five to 10 min later 20 μl. 20% unlabelled ferrocyanide solution were rapidly injected as a pulse into the aorta.

After 8–10 sec, the time being precisely measured in each case, the left renal pedicle was tied and an arterial blood sample was collected. The left kidney was then removed, frozen and the ferrocyanide was precipitated in alcoholic ferric chloride solution at $-20°$. After maceration, fifteen complete superficial and juxtamedullary proximal tubules were dissected and cut at the front level of the visible blue unlabelled precipitate. These contain the [^{14}C]ferrocyanide filtered during the 8–10 sec period. SNGFR was then calculated by determining the plasma [^{14}C]ferrocyanide radioactivity and [^{14}C]ferrocyanide precipitate contained in the proximal tubule segment upstream of the front.

TABLE 1. Comparison between inulin and ferrocyanide clearance in the young rat. C_{Fe}/C_{in} = relative clearance of ferrocyanide. Values are means ± S.E.

Age (days)	Body weight (g)	Inulin clearance (μl./min)	Ferrocyanide clearance (μl./min)	$\frac{C_{Fe}}{C_{in}}$
38 ± 4 (8)	79 ± 8 (8)	562 ± 65 (8)	535 ± 52 (8)	0·97 ± 0·04 (8)

Validity of the [^{14}C]ferrocyanide infusion technique for measurement of SNGFR in maturing rats

The use of the [^{14}C]ferrocyanide infusion technique in this study depends on the assumption that in the immature, growing rat, the technique provides as good a measure of SNGFR as in the adult. This has been checked in two ways.

Firstly, in eight rats aged between 25 and 59 days, [^{3}H]inulin and [^{14}C]ferrocyanide clearances were compared. The ferrocyanide plasma concentration was raised above 1 m-mole/l., as was done in the experiments where SNGFR was measured. As shown in Table 1 the [^{14}C]ferrocyanide: [^{3}H]inulin clearance was 0·97 ± 0·04. This ratio is in complete agreement with the ratio obtained in the adult rat (Bonvalet, Bencsath & Rouffignac, 1972).

Secondly additional experiments were performed in order to ensure that, as has been demonstrated in the adult rat (see full details in Chabardes, Poujeol, Deiss, Bonvalet & Rouffignac, 1974), the radioactive count per nephron fragment derives almost entirely from intraluminally located ferrocyanide, and that any extraluminal contamination is relatively small. However, it might be objected that such extraluminal contamination could be different in the young rat compared with the adult rat. For this reason 30 μl. of a 15% [^{14}C]ferrocyanide solution (300 μc) were injected as a pulse into the abdominal aorta, just above the renal arteries as previously described (Rouffignac & Bonvalet, 1970), of two young rats (22 and 24 days old, 60 and 75 g body weight) receiving a saline infusion (0·9% NaCl, 15

μl/min. 100 g). Eight seconds after the pulse injection the left renal pedicle was tied. The kidney was then removed and treated as indicated above to precipitate ferrocyanide. Forty-five proximal and twenty-four distal tubules were microdissected and their radioactivity was counted. Under these experimental conditions, distal tubular lumen should contain no radioactivity, and any distal tubular counts represent extraluminal contamination which is assumed to be similar to extraluminal contamination of other cortical nephron fragments. For the proximal tubules, the ^{14}C radioactivity per mm of proximal tubule containing the radioactive prussian blue precipitate was $28{\cdot}4 \pm 2{\cdot}1$ c.p.m./mm. For the distal tubule the ^{14}C radioactivity per mm of distal tubule amounted only to 4·6% of the former value ($1{\cdot}3 \pm 0{\cdot}1$ c.p.m./mm). This value is quite similar to the value found in the adult rat, in which the same kind of verifications have been made (Chabardes *et al.* 1974).

Analytical procedures

[^{3}H]inulin radioactivity was measured in a liquid scintillation counter (Tri-Carb Packard) in 10 ml. aliquots of Bray solution containing 3% Cab-O-Sil. The ^{14}C radioactivity was measured in a gasflow counter (Nuclear Chicago).

Statistical procedures

The statistics were done according to Snedecor's methods. Means are given with their standard deviation (S.D.) or their standard error ($S/\sqrt{N}$) and Student's t test was used to evaluate the statistical significance of differences between the means. Correlation coefficients and equations of regression lines were calculated by the least-squares method.

RESULTS

As expected, kidney weight the number of nephrons and inulin clearance all increased significantly with age (Table 2). However, since most of the experimental data were more closely correlated with body weight than with age, the results will be considered in relation to changes in body weight.

As seen in Fig. 1 the mean arterial blood pressure increased significantly with body weight from 90 ± 5 mmHg in the 40–60 g rats to 125 ± 8 mmHg in the adult rats.

The increase in kidney weight was relatively less than that in body weight. This increase was attributable both to the increase in the number of nephrons (Table 2) and to structural changes in the glomerular and tubular components of individual nephrons as manifested by increases in glomerular volume and tubular length in both JM and S nephrons (Table 3). The increases in glomerular volume were relatively greater than those in tubular length for both JM and S nephrons. At all ages both glomerular volume and tubular length were smaller in S than in JM nephrons. The S/JM ratio for both the glomerular volume and the tubular length were not correlated with body weight (Fig. 2). This ratio was constant and averaged $0{\cdot}72 \pm 0{\cdot}12$ S.D. and $0{\cdot}81 \pm 0{\cdot}05$ S.D. respectively.

The increases in inulin clearance (approximately tenfold, Fig. 3) were

TABLE 2. Changes in body weight, kidney weight, number of nephrons and GFR as a function of age in rats during growth

Rat	Age	Body weight (g)	Weight of the right kidney (mg)	Number of nephrons right kidney	Clearance of inulin ml./min left	right
EG	23	36	241	20,250	0·222	0·203
EH	24	40	278	15,250	0·180	0·154
EC	23	42	316	14,250	0·198	—
EM	25	46	227	17,250	0·272	0·260
EB	25	61	410	20,250	0·283	0·316
EI	30	74	373	17,250	0·605	0·635
EK	33	74	389	16,750	0·732	0·739
ED	32	85	431	15,500	0·740	0·799
EL	32	93	450	11,250	0·719	0·747
EF	30	105	548	24,250	0·896	1·219
EE	41	120	542	—	1·043	0·976
ER	61	210	772	34,250	1·682	1·671
EN	65	218	872	30,000	1·633	—
EO	84	227	848	23,500	1·604	1·653
EP	91	275	796	31,250	1·319	1·449

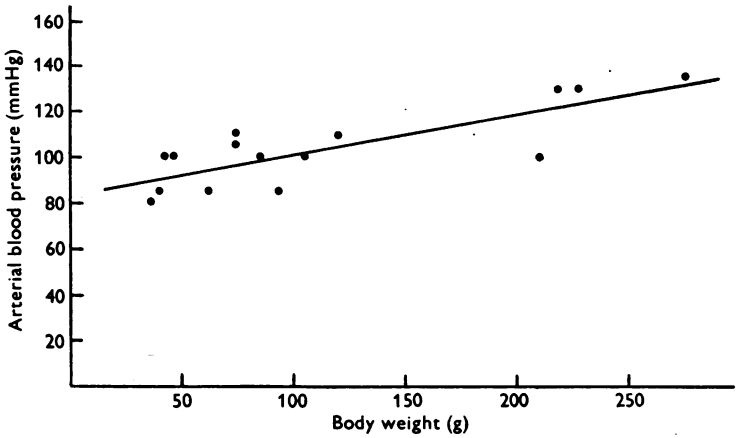

Fig. 1. Increase of mean arterial pressure as a function of body weight in rats during growth. Continuous line = regression line. $y = 0{\cdot}176x + 84$, $r = 0{\cdot}81$, $P < 0{\cdot}01$.

associated with functional increases in SNGFR (Table 3) as well as the increased number of nephrons. The rate of increase was greater for the superficial nephrons as indicated in Fig. 4 in which the superficial over juxtamedullary SNGFR ratio is related to body weight. This ratio increased from 0·60 in the 40–60 g rats to 0·84 in the adult rats. These data demonstrate the existence of the expected centrifugal functional maturation of the nephrons. At all ages SNGFR was higher in JM than in S nephrons.

TABLE 3. Anatomical and functional data obtained for superficial (S) and juxtamedullary (JM) nephrons in rats during growth. Values are means ± S.E.

Rat	Age (days)	Weight (g)	Length of the proximal tubule (mm) S	Length of the proximal tubule (mm) JM	Glomerular volume (nl.) S	Glomerular volume (nl.) JM	SNGFR (nl./min) S	SNGFR (nl./min) JM
EH	24	40	3·5 ± 0·5	4·5 ± 0·6	0·29 ± 0·07	0·40 ± 0·10	13·9 ± 5·6	22·4 ± 4·6
EC	23	42	3·6 ± 0·5	4·5 ± 0·8	0·23 ± 0·05	0·35 ± 0·14	21·4 ± 3·6	34·5 ± 10·5
EM	25	46	2·9 ± 0·4	3·8 ± 0·6	0·24 ± 0·11	0·29 ± 0·11	12·0 ± 5·1	21·6 ± 8·9
EB	25	61	3·9 ± 0·6	4·5 ± 0·9	0·36 ± 0·10	0·42 ± 0·17	15·5 ± 4·3	24·4 ± 10·3
EI	30	74	4·3 ± 1·0	5·6 ± 0·7	0·33 ± 0·10	0·58 ± 0·28	30·8 ± 6·3	41·4 ± 18·9
EL	32	93	4·3 ± 0·7	5·2 ± 0·4	0·27 ± 0·07	0·44 ± 0·15	27·0 ± 5·3	38·8 ± 9·8
EF	30	105	5·8 ± 0·9	6·9 ± 0·8	0·51 ± 0·23	0·66 ± 0·18	29·7 ± 8·5	42·3 ± 12·0
EE	41	120	6·9 ± 1·3	8·7 ± 1·4	0·77 ± 0·15	1·25 ± 0·33	34·7 ± 7·4	43·7 ± 14·1
ER	61	210	6·6 ± 0·9	7·8 ± 1·0	1·00 ± 0·31	1·39 ± 0·46	47·8 ± 12·0	55·3 ± 19·5
EN	65	218	7·7 ± 1·4	9·7 ± 1·0	1·17 ± 0·46	1·73 ± 0·68	50·7 ± 10·4	61·0 ± 12·9
EP	91	275	6·2 ± 0·4	6·6 ± 0·7	1·16 ± 0·44	1·17 ± 0·36	36·8 ± 6·2	44·0 ± 7·4

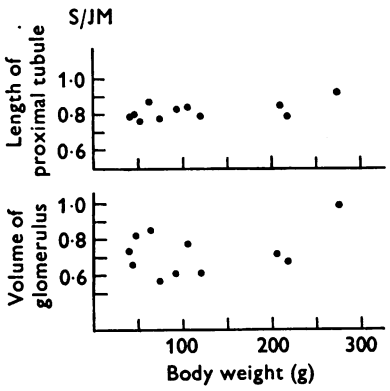

Fig. 2

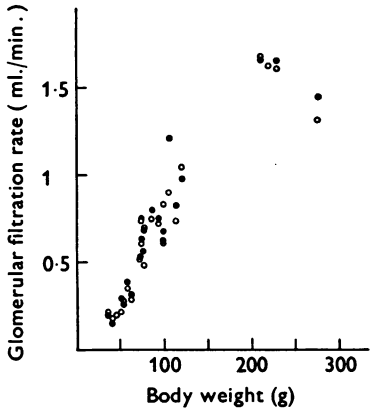

Fig. 3

Fig. 2. Lack of correlation between the S/JM ratio for the length of proximal tubule (upper panel) or glomerular volume (lower panel) and body weight, in rats during growth. Each value was obtained by dividing mean S values by mean JM values obtained in each rat.

Fig. 3. Increase of GFR with body weight in rats during growth ○, left kidney; ●, right kidney. In this Figure are plotted the data obtained in the twenty-three rats used in this study.

For both JM and S nephrons, the increases in SNGFR were significantly correlated with glomerular volume and with proximal tubular length ($P < 0·01$ in all cases).

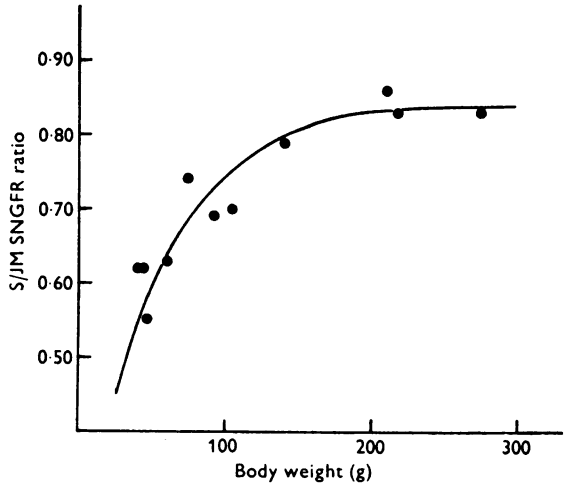

Fig. 4. Increase of the S/JM SNGFR ratio as a function of body weight in rats during growth. Each value was obtained by dividing the mean S SNGFR by the mean JM SNGFR in each rat. The continuous line was drawn free hand.

DISCUSSION

The [^{14}C]ferrocyanide infusion technique as used in the present study was chosen because it is based on two principles which are important from a methodological point of view: the radioactive indicator is continuously infused into the animal, and the SNGFR is determined at a plasma ferrocyanide concentration which is constant and always above the value required for ferrocyanide to behave as a glomerular indicator. Neither of these two conditions are adequately met when the ferrocyanide is injected as a pulse, as it was demonstrated that this could lead in certain circumstances to over-estimation of the superficial nephron SNGFR (Chabardes, *et al.* 1974), nor when the indicator is infused into the animal only during a short period of time, as was proposed by Coelho, Chien & Bradley (1972).

The ratio clearance of ferrocyanide:inulin,and the percent of extraluminal contamination along the tubule fragments in the young rat were quite similar to those obtained in the adult rat, indicating that the technique gives a valid estimate of SNGFR during maturation.

This study reports that the mean arterial blood pressure increases in the growing animal. This observation has already been described in the rat (Aperia & Herin, 1975; Horster & Lewy, 1970), as well as in the dog (Horster & Valtin, 1974; Kleinman & Reuter, 1973; Olbing *et al.* 1973), the guinea-pig (Merlet-Benichou & Rouffignac, 1974; Spitzer & Edelmann, 1971), and the lamb (Aperia, Broberger & Herin, 1974).

Concomitantly, it was observed that the glomerular filtration rate (GFR)

rose rapidly as a function of body weight. Only a small part of the rise in GFR can be explained by the increase in the number of nephrons which occurs in the rat after birth (Arataki, 1926; Baxter & Moffey, 1948; Bonvalet, Champion, Wanstok & Berjal, 1972; Goncharevskaya & Dlouha, 1975; Jamison, 1972). In fact this rise is mostly due to increase of SNGFR during growth. Which factor(s) can explain this increase of SNGFR during growth?

In the mature rat the oncotic pressure in the glomerular capillaries of superficial nephrons has risen to equal the hydrostatic pressure before the end of the glomerular capillary (Brenner, Troy, Daugharty, Deen & Robertson, 1972; Deen, Robertson & Brenner, 1972). As a consequence of the attainment of this filtration pressure equilibrium, a high degree of dependence of SNGFR on plasma flow in the adult rat can be predicted (Deen, Troy, Robertson & Brenner, 1973). The same dependence was recently found in the immature rat by Aperia & Herin (1975). In rats between 17 and 60 days old these authors found a constant relationship between SNGFR of the superficial nephrons and glomerular perfusion rate. They concluded that the glomerular perfusion rate was the major factor responsible for the increase of SNGFR with growth. It is possible that age-dependent changes of the effective filtration pressure and/or the filtration surface and/or the hydraulic permeability of the filtration membrane are necessary to allow the maintenance of filtration pressure equilibrium during growth. In this respect Allison *et al.* (1972) calculated that the effective filtration pressure at the beginning of the glomerular capillaries increased from 21·7 in young to 25·5 mmHg in adult rats. Our study demonstrates that glomerular volume in the adult rat is about 4 times greater than in 40–60 g rats. It is also probable that in the rat as well as in the guinea-pig (Spitzer & Brandis, 1974) and man (Osathanondh & Potter, 1966) for a given glomerular volume an increase in the area occupied by glomerular capillaries occurs. No data are known about the development of the effective hydraulic permeability of the glomerular capillary membrane in the rat. In the human kidney however, it has been shown by Arturson, Groth & Grotte (1971) that a gradual increase in permeability does occur as a developmental phenomenon.

As far as juxtamedullary nephrons are concerned, not enough direct data are available to discuss with assurance the increase of SNGFR during maturation. On the basis of indirect arguments however, it was recently concluded that SNGFR of both superficial and juxtamedullary nephrons are controlled by similar factors, since in most situations examined the SNGFR changes were strictly parallel for the two nephron categories (Rouffignac, 1975).

The present work demonstrates that in the 3–9 weeks old rats the ratio

superficial over juxtamedullary glomerular volume was constant, irrespective of the age of the animal (see Fig. 2). This observation was also reported by Horster *et al.* (1971) in the dog during the same period of life. Thus, even if there was a close relationship between the development of glomerular volume (or proximal tubular length) and that of SNGFR in both nephron categories of the young rat, it appears that the greater rise in SNGFR for S than for JM nephrons was not accompanied by disproportionate changes in glomerular volume (or proximal tubular length). Whatever the explanation might be, Fig. 4 clearly shows that the ratio superficial SNGFR: juxtamedullary SNGFR increases during growth in the rat. These data are in good agreement with the results recently reported by Dlouha, Bibr Jezek & Zicha (1975). Using Hanssen's (1963) technique they noted an increase in this ratio from 0·52 in 20 days old rats to 0·73 in 40 days old rats and to 0·90 in 60 days old rat.

These observations indicate that the functional maturation of the nephrons continues from the juxtamedullary region towards the periphery during post-natal life in the rat. Such a conclusion has already been drawn for the dog (Horster & Valtin, 1974) and the guinea-pig (Spitzer & Brandis, 1974).

We gratefully acknowledge the skilful technical assistance of M. S. Deiss in the preparation of this paper.

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