ACTA STEREOL 1990; 9/2: 243-258
ORIGINAL SCIENTIFIC PAPER

A PRACTICAL METHOD TO COUNT THE NUMBER OF GLOMERULI IN THE KIDNEY AS EXEMPLIFIED IN VARIOUS ANIMAL SPECIES

Jens Randel Nyengaard and Thomas Fichtner Bendtsen

Stereological Research Laboratory, University Institute of Pathology and 2nd. University Clinic of Internal Medicine, Institute of Experimental Clinical Research, Aarhus University, Denmark

ABSTRACT

A new stereological counting method, the fractionator, is applied in several animals to count the number of glomeruli. The details of the method are described for use in a small animal (rat) and in human autopsy kidneys. When the number of glomeruli was counted in animals with a wide spectrum in body weight, it was observed that glomerular number correlates closely with body weight and kidney weight. The method is recommended for anatomical and pathological studies when a precise knowledge about the number of glomeruli is needed.

Keywords: body weight, fractionator, glomerular number, kidney, stereology.

INTRODUCTION

Counting of renal glomeruli has been performed for more than a hundred years by means of three different principles — serial sections, independent sections and maceration technique — as recently reviewed by Bendtsen and Nyengaard (1989).

The only unbiased application of ***serial sections*** was performed by Kittelson (1917), Arataki (1926a, 1926b) and Jackson and Shiels (1927). These authors counted all glomeruli using complete cross sections. Complete microscopical sections cannot be made in larger kidneys (e.g. human), and the workload of counting all glomeruli is almost prohibitive.

Counting glomeruli on ***independent sections*** has been performed by Elias and Hennig (1967). This method was similar to Saltikov's (1967), which was a practical application of Wicksell's (1925) principle. McLachlan et al. (1977) counted glomeruli on independent sections ad modum Weibel and Gomez (1962) . Eisenbrandt and Phemister (1980) counted glomeruli with both methods. Both methods are biased by model assumptions assuming constant shape, and Weibel and Gomez's method furthermore make rather strong assumptions about the size distribution. The last method is also biased

by the positive thickness of sections. Both methods are biased by dimensional changes during preparation, overprojection and lost caps (Cruz-Orive, 1983). All the methods deducing number from observations on independent sections are inefficient - because the estimates are indirect, and the stereological transformations are statistically unstable (Cruz-Orive and Hunziker, 1986).

Isolation and counting of glomeruli by *maceration* in hydrochloric acid was invented by Schweigger-Seidel (1865). Intraarterial in vivo injection of Janus Green B was made by Nelson (1922). Vimtrup (1928) modified this method and made in vitro injection of Prussian Blue before maceration. Kunkel (1930) made his estimate on volume-weighted fractions, and his method was used by all modern authors employing maceration, based on Damadian et al. (1965). The latest example is Larsson et al. (1980). The method is biased by overmaceration, cutting up of glomeruli (when the kidney is cut to pieces) and incomplete injection. The sum of biases can be quite large: By application of practically identical methods on normal human kidneys the results of Moritz and Hayman (1934) and Moore (1931) differed by a factor of two!

As described there are numerous problems in obtaining a correct estimate of the number of glomeruli in a kidney and none of the many above mentioned methods allow the unbiased counting of glomeruli in just *fractions* or samples of a kidney. If all glomeruli in a kidney are counted, it represents an overwhelming amount of work, no matter which method is used. Instead the workload can be minimized by counting glomeruli in a known fraction of the kidney (*the fractionator principle*, Gundersen, 1986). The estimate is *unbiased* because the mean of all possible estimates is identical to the *true value*. Secondly, the method is *efficient* because of the minimal workload necessary to obtain a low variability of the estimate.

To exemplify our practical application of the fractionator principle, we have counted glomeruli in various animals. The kidneys of vertebrates excrete metabolic waste products by means of the filtration-reabsorption principle with tubular secretion added (Schmidt-Nielsen, 1975). We have observed, that the size of glomeruli among species is almost invariant. There is a close relationship between resting metabolic rate and the body size of animals (Schmidt-Nielsen, 1984). We wanted to investigate whether the larger load of metabolic waste products on the kidneys from larger animals is eliminated by means of a larger number of glomeruli. Therefore we examined the number of glomeruli in a range of animals with body weight varying by five orders of magnitude.

In order to get an impression of the variation of size of glomeruli among mammals, we estimated the size of glomeruli in the smallest and largest mammal, i.e. a mouse and a pilot whale.

MATERIAL

The basic idea of species selection was to obtain as large a spread as possible on body surface area. The animals were sampled arbitrarily, when the opportunity was present. Only kidneys from mature animals which were normal at the macroscopical and microscopical level were used. All kidneys were immersion fixed, except for the rat kidney, which was perfusion fixed. The choice of the sex of the

animal and of right/left kidney was arbitrary, but supposedly close to random because of the big spread of sources.

METHODS

The kidney weight for each animal was measured after fixation.

The fractionator principle: Take a complete kidney and section it in any arbitrary way into a number of pieces, e.g. 10. *Sample* a predetermined *fraction* of the pieces (1/4, say) at *random*, i.e. take the first piece at random from the first four (no. 2, say) and every fourth thereafter, i.e. numbers 6 and 10 as well. Count the glomeruli in the sampled pieces, N'. An *unbiased estimate* of the total number N of glomeruli in the whole kidney is

$$N = 4 \cdot N' \qquad (1)$$

Because of the unbiasedness, it is only a matter of workload to obtain a precise estimate of the true value. However, the described method is relatively inefficient. *Efficiency* is here defined as "amount of time spend to obtain a low variability". The efficiency can be improved in several ways. Firstly, the pieces can be sectioned in roughly *equal sizes.* Secondly, in an organ like the kidney the sampled pieces, with *kept mutual order* at each sampling step, should be disposed in a *dome pattern*, e.g. the size of the sampled pieces will increase smoothly from each end towards the middle of the sampling series (Ogbuihi and Cruz-Orive, 1990). Finally, since the distribution of glomeruli in the kidney is inhomogenous, sampling of an *adequate number* of pieces should be *systematical*, as described in a stereological context by Gundersen and Jensen, 1987.

The actual counting is performed using the so-called disector counting principle (Sterio, 1984), explained below.

Macroscopical fractionation of the kidney: A fraction of the kidney is chosen by systematic sampling of pieces from the removed kidney. The sampled tissue pieces are embedded and then cut in thin sections on a microtome. The embedding is made in ordinary capsules. To obtain kidney pieces sufficiently small to fit into these capsules, it is necessary to fractionate large and small kidneys in different ways. This is illustrated below by two examples.

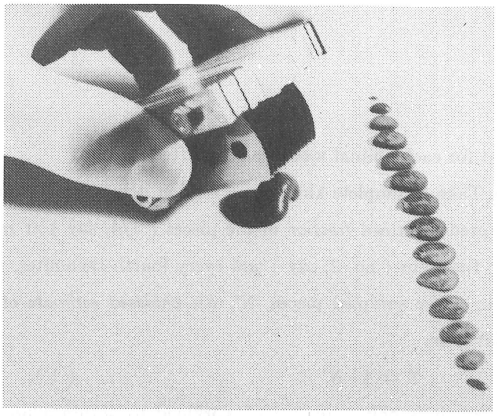
(A) *Rat kidney*, weight: 1.12 grams.

After perfusion fixation the kidney is cut into 1.5 mm slices in the horizontal plane by means of a razor blade tissue slicer (Fig. 1) as described by Baddeley et al. (1986).

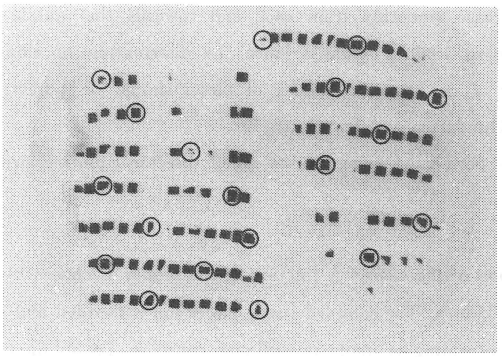
From the series of slices, every third is sampled systematically, i.e. $f_1 = 1/3$. The first block is chosen by means of a random number between 1 and 3. In total about 4 slices should be sampled.

(B) *Human kidney*, weight: 181 grams.

Before immersion fixation, the kidney is split along the frontal plane into two halves, which are



(Fig.1) The razor blade tissue slicer (Baddeley et al., 1986) which has been applied for cutting the rat kidney into parallel 1.5 mm thick slices.



(Fig.2) The third step of sampling from the human kidney is shown: The agar slices are arranged in two columns in a Petri dish - each agar slice contains small tissue blocks. One seventh of the *tissue blocks* is sampled by means of the "modulus" system: A random number is looked up between 1 and 7, to decide which of the first seven blocks in the first row to be sampled. After the sampled block every seventh block in the same row is sampled. In the *next* row, the sampling of blocks is displaced three steps to the right (or four steps to the left), compared to the sampling positions in the foregoing row. The displacement is equal to the integer of half the reciprocal sampling fraction: $\frac{7}{2} = 3.5 \Rightarrow 3$. The denominator in the sampling fraction must be odd, the numerator is also odd, namely 1. This system corresponds to a skewed lattice of frames, where a seventh of the frames are predetermined for sampling, when the number of the first block to be sampled has been decided. Of course, more simple methods of sampling do not violate unbiasedness (Gundersen, 1986, Ogbuihi and Cruz-Orive, 1990), but the modulus system makes the sampling of the blocks from the kidney uniform and almost 'equidistant' among the strips by minimizing the risk of sampling from strips, which are close to each other in the kidney.

fractionated separately. On a slicing machine both halves are exhaustively cut into 4 mm thick slabs in the horizontal plane of the kidney. A quarter of all slabs in the two halves are sampled systematically, i.e. $f_1 = 1/4$. In the first half, the first slab is chosen by means of a random number (R) between 1 and 4, after which every fourth slab is chosen. In the other kidney half, the first slab is chosen by R shifted by 2: If R is 1, number 3 is used in the second half, whereas if R 4, number 2 is used in the second half. Medulla is cut out from the sampled slabs, but a rim of medulla is kept, to asure that no glomeruli are excised. The arcuate arteries constitute the border between medulla and cortex (Heptinstall, 1983). The sampled slabs are placed side by side in their natural order and then embedded in agar. The embedded slabs are cut in the sagittal plane of the kidney into 5 mm strips.

From the agar slabs every fifth tissue strip is sampled systematically ($f_2 = 1/5$) by means of a "modulus" system (see Fig.2). The first strip is chosen by means of a random number table.

The sampled strips are embedded side by side in agar and cut perpendicular to their length in 1.5 mm agar slices. These agar slices contain 4 by 5 mm kidney blocks of 1.5 mm thickness. Every seventh kidney block is sampled ($f_3 = 1/7$) by means of the modulus system (Fig. 2). The number of first block is chosen by a random number table. In total, from 30 to 40 blocks should be sampled.

The macroscopical fractionation of the human kidney can be performed either by cutting with a sharp knife, with an ordinary slicing machine or with a specially designed chopping machine (Fig. 24, Gundersen and Jensen, 1987). As long as sampling is random, the result becomes unbiased. But efficiency is improved, when the thickness of the slabs, strips and blocks at each sampling level is as constant as possible. The embedding in agar can be done on wax plates, perforated by pinpricks. The wax plates stabilize the cutting of the agar blocks. To avoid dessication, the sampling units are placed in buffer in Petri dishes during processing.

Microscopical fractionation of the kidney: Dehydration of the sampled blocks was performed using ordinary methods. Infiltration and embedding was done with JB-4 glycolmethacrylate (Polysciences Data sheet 123B). The polymerized plastic blocks were serially sectioned at 15 μm on a microtome (2218 Historange Microtome, LKB) with carbide tungsten disposable metal knives (LKB no. 2218-521). We added 5% polyethylenglycol 400 (PEG 400) to the infiltration solution to soften the embedded tissue, which made the cutting of the thick sections possible. Using glass Ralph-knives it is possible to cut 25 – 50 μm plastic sections. The counting is less time consuming with increasing section thickness.

(A) *Rat kidney.*

During sectioning, every tenth section is sampled ($f_2 = 1/10$) as well as the look-up section. The first section is chosen using a random number between 1 and 10. In the first two steps $\frac{1}{3} \cdot \frac{1}{10} = \frac{1}{30}$ of the kidney has been sampled.

(B) *Human kidney.*

Every tenth section is sampled ($f_4 = 1/10$). To summarize, until now $\frac{1}{4} \cdot \frac{1}{5} \cdot \frac{1}{7} \cdot \frac{1}{10} = \frac{1}{1400}$ of the renal cortex has been sampled (not including the look-up sections).

The sections were stained with PAS.

Counting of glomeruli: The counting is carried out in a dark room with two ordinary light microscopes (Zeiss) adapted to projection with a halogen light source. The fields of vision are projected side by side onto a table using a projection mirror in front of the monocular tube. A grid is applied to the field of vision of the first microscope (Fig. 3).

A slide with a sampled histological section is placed in the first microscope and the corresponding look-up section in the second microscope. The stage of the first microscope is moved by a motor in predetermined, equidistant steps in two orthogonal directions. In this way, the fraction of the histological section covered by the test system is well defined: These steps constitute the final sampling step (f_a = areal fraction).

In the rat kidney, the total grid area is 59731 mm^2 and the *final linear* magnification is 115×, which means that the area of the section covered by the grid is ($59731/115^2$) $mm^2 = 4.52\ mm^2$. The motor is adjusted to step 5.12 mm along the length of the microslide and 4.90 mm orthogonal to the length of the microslide. Consequently the fraction of the section area which is covered by the grid moved in discrete steps is: $f_a = \frac{4.52}{5.12 \cdot 4.90} = 0.18$.

A glomerulus is counted if it appears in a sampled field of vision, and is *not present in the look-up section* — defined as Q^- (Fig.4). *It is prerequisite that any glomerulus unambigously can be identified in at least one section in the section series.* To control this, we made a double estimate for the duck at x76 and x190 magnification, and found no difference: 423.000 compared to 439.000 glomeruli.

When the kidney was cut into pieces before the embedding, the knife produced artificial surfaces (the cutting plane). When an embedded tissue block is sectioned, artificial edges of the section are produced where the microtome hits the artificial surfaces of the embedded tissue block. A fraction of the sections covered by the visual fields is excluded for glomerular counting because it is too close to either artificially cut edges (Fig.3) or surfaces (Fig. 4), where glomerular identity is ambigous or glomeruli may be lost, when glomeruli are hit by the knife.

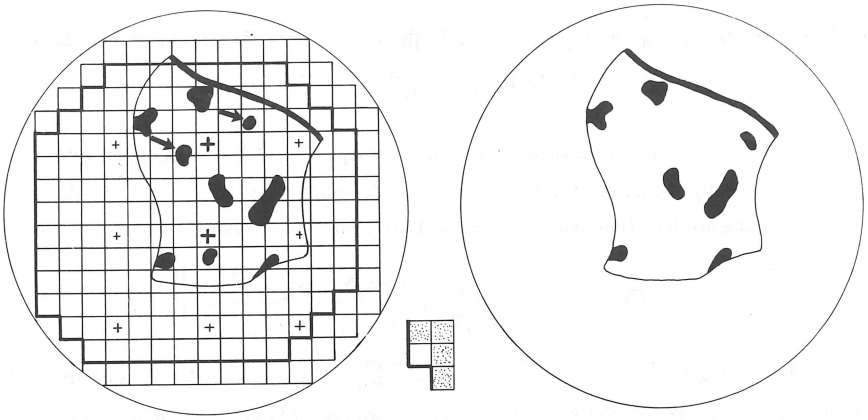
The fraction of tissue used for counting is estimated by $\frac{P_f}{P_s}$ (see Fig. 4 for estimation of P_f and P_s).

The general formula of estimation of particle number using the fractionator, m denotes sections:

$$N = \frac{1}{f_1} \cdot \frac{1}{f_2} \cdot \ldots\ldots \cdot \frac{1}{f_n} \cdot \frac{1}{f_a} \cdot \frac{P_s}{P_f} \cdot \sum_{i=0}^{i=m} Q^-_i \qquad (2)$$

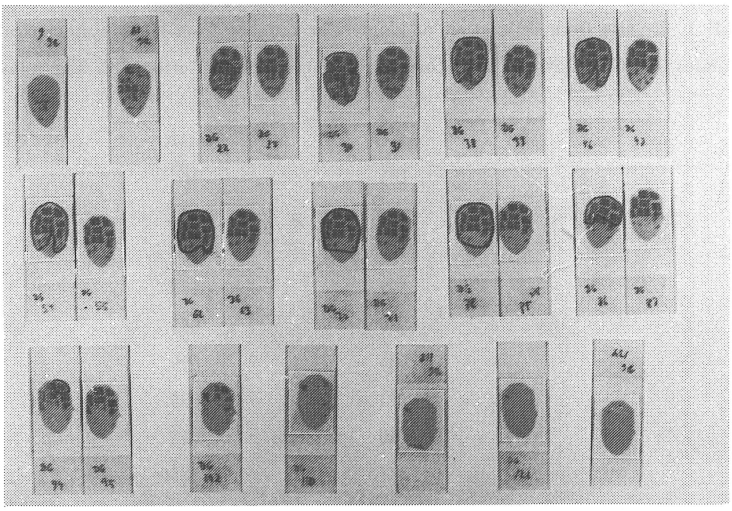
(A) *Rat kidney:* $f_1 = \frac{1}{3}$, $f_2 = \frac{1}{10}$, $f_a = 0.18$, $P_s = 87$, $P_f = 58$, $\sum Q^- = 102$

$$N = 3 \cdot 10 \cdot \frac{1}{0.18} \cdot \frac{87}{58} \cdot 102 = 25{,}500 \text{ glomeruli.}$$



(Fig.3) The drawing shows a visual field on a sampled tissue section and the corresponding visual field on the look-up section. A grid is superposed on the visual field hitting the sampled section. A glomerular *profile* is sampled in a frame in the lattice if the two criteria of the unbiased 2-D counting rule (Gundersen, 1977 and 1986) – illustrated among the two fields are fullfilled: (1) The profile is contained completely or partly in the frame and is *not* hit by the fully drawn exclusion line (2) No *artificial* edges are present in the frame or in its guard area (corresponding to the dotted area).

The heavily drawn, curved tissue edge is a natural surface. Only two of the *particles* are counted (indicated by arrows) because their black profiles are sampled in the sampled section, according to the 2-D counting rule, and they are *not* present in the look-up section. Q^- is therefore 2. The crosses are used to estimate the P_f/P_s fraction (the fraction of tissue permitted for counting glomeruli): One counts crosses hitting any tissue (P_s), and crosses hitting tissue surrounded by a sufficient guard area (P_f). In the example $P_s = 4$ and $P_f = 2$, the two P_f points are shown heavily drawn.



(Fig.4) The figure shows sampled and look-up sections from one plastic block from a human kidney. Every tissue block is followed through the whole section series. The first and the last section containing the whole circumference of the block is found. All the sections of the block between these two sections are circumscribed. P_f and Q^- values can *only* be counted inside circumscribed sections. Sections not circumscribed have been cut close to artificial surfaces, and are then excluded for the counting P_f and Q- values, because glomerular identity is ambigous or glomeruli may be lost. However, they are included for the counting of P_s – see text.

(B) *Human kidney:* $f_1 = \frac{1}{4}$, $f_2 = \frac{1}{5}$, $f_3 = \frac{1}{7}$, $f_4 = \frac{1}{10}$, $f_a = 0.37$, $P_s = 213$, $P_f = 128$, $\sum Q^- = 114$

$$N = 4 \cdot 5 \cdot 7 \cdot 10 \cdot \frac{1}{0.37} \cdot \frac{213}{128} \cdot 114 = 718{,}000 \text{ glomeruli.}$$

The efficiency of the method was tested according to equation 17 in Cruz-Orive (1990), based on eigth systematically sampled, double estimates as described in Gundersen, 1986. As we take in consideration the artificial edges and surfaces, eq. 17 in Cruz-Orive (1990) is adjusted to our results:

$$\overline{CE}\ (N) = \left[\frac{1}{3n} \cdot \sum_{i=1}^{n} \left(\frac{(est_1N_i - est_2N_i)^2}{(est_1N_i + est_2N_i)^2} \right) \right]^{\frac{1}{2}} \qquad (3)$$

This is the 'average' coefficient of error, CE ($= \frac{SEM}{mean}$), of one animal estimated from n animals (table 1). CE of the method was 4.5 per cent. On average, 135 glomeruli were counted per kidney, which means that the expected CE was $\frac{1}{\sqrt{135}} = 8.6$ per cent. The very small variation compared to the expected CE of 8.6 per cent means that the method, in particular the systematic sampling, *per se* is *very* efficient. It is not necessary to count more than 100 glomeruli per animal. Other results (Nyengaard and Bendtsen, submitted) are in accordance with this, e.g. counting 107 glomeruli in 25 human kidneys with a CE of 4.8 per cent.

TABLE 1: Results from estimation of glomerular number from eigth systematically sampled, double estimates, est_1N, est_2N and their corresponding coefficient of error, CE. The numbers refer to table 2.

N^O	est_1N 10^3	est_2N 10^3	CE (N) %
3	15.7	14.6	2.1
13	166	179	2.2
14	172	173	0.2
23	530	665	6.5
24	584	599	0.7
25	784	655	5.2
26	750	912	5.6
29	3772	4850	7.2
		Mean CE (N)	4.5

Estimation of mean volume, $\bar{v}_N$, of glomeruli per animal: The general formula is:

$$\bar{v}_N = \frac{V_V}{N_V}. \quad (4)$$

V_V is equal to the volume fraction of glomeruli in the reference space, and N_V is equal to the number of glomeruli in the reference space. V_V is estimated by point-counting, see for instance Gundersen et al., 1988.

$$N_V = \frac{\sum Q^-}{a(p) \cdot t \cdot \sum P_f} \quad (5)$$

a(p) is the area corresponding to one point used for P_f estimation in the grid, i.e. the total area of the grid divided with number of points used for P_f estimation, and t is the section thickness. Eq. 5 is the numerical density estimate using the disector (Sterio, 1984).

Statistics: We have applied the F-test with a level of significance of 0.05 in the comparison of the slopes from the correlation lines.

RESULTS

Animal and kidney weights are shown in table 2 together with individual estimates of the number of glomeruli. The results below only include mammals.

The log-log correlation between number of glomeruli and body weight (BW) among species is significant (Fig.5). The slope is 0.67 (± 0.039, SEM) which is identical to the slope (2/3) of the general relation of body surface area (BSA) against BW. The correlation between basal metabolic rate (BMR) and BW has a slope of 0.75, see the review by Schmidt-Nielsen, 1984. There was no significant difference between this value of 0.75 and the observed slope of 0.67 ($0.05 < 2p < 0.1$). The correlation between BW and kidney weight (KW) is also highly significant, $r = 0.98$. The slope 0.93 (± 0.042) does not differ significantly from 1.00 ($2p > 0.05$), but is clearly above the slopes of 0.67 ($2p < 0.05$) and 0.75 ($2p < 0.05$).

The number of glomeruli varied by a factor of 3000 from mouse (8.600) to pilot whale (25.700.000), whereas the BW varied by a factor of 40.600 from mouse (40.7 g) to pilot whale (1652 kg).

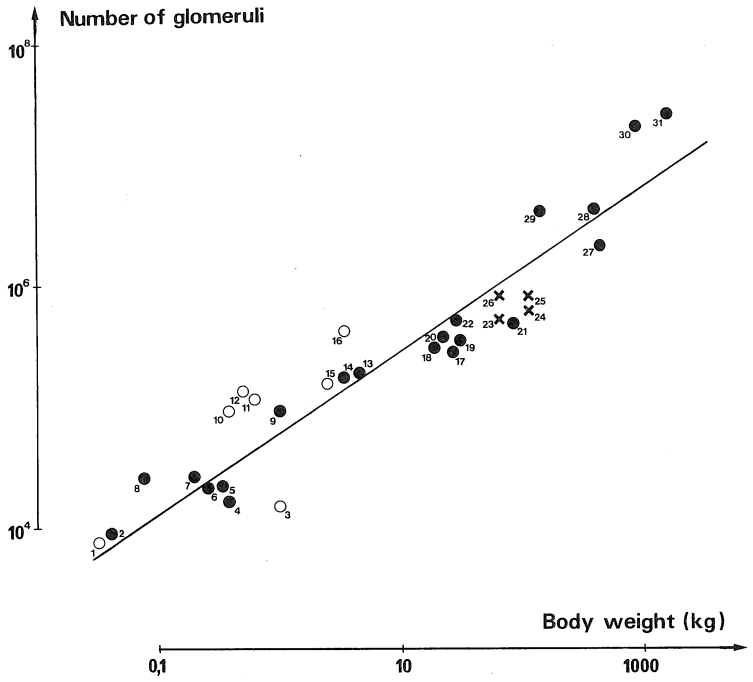
The mean glomerular volume ($\bar{v}$) differed by only a factor of 7 from mouse ($1.3 \cdot 10^6 \mu m^3$) to pilot whale ($9.5 \cdot 10^6 \mu m^3$).

DISCUSSION

The applied fractionator principle has several advantages: It is simple and does not involve much work. Futhermore the method is unbiased and without model assumptions. The only risk of bias is an *ambigous level of identification.* If, for instance, the observation of the look-up sections is less

TABLE 2: Species, kidney weight, body weight and the number of glomeruli of the sampled animals.

N^{O}	SPECIES	KIDNEY WEIGHT	BODY WEIGHT	N(GLOM)
		g	kg	10^3
1	frog, *esculenta rana*	0.08	0.033	7.34
2	mouse, *mus musculus*	0.22	0.041	8.60
3	turtle, *pseudemys scripta*	2.1	1.00	15.1
4	rat, *rattus norvegicus*	0.81	0.31	19.8
5	rat	0.94	0.28	23.2
6	rat	0.81	0.25	23.5
7	rat	1.12	0.21	25.5
8	mole, *talpa europaea*	0.26	0.073	26.0
9	hedgehog, *erinaceus europaeus*	1.83	1.04	93.0
10	trout, *salmo gairdneri*	6.16	0.373	94.0
11	trout	7.43	0.580	123
12	trout	8.34	0.512	168
13	wallaby, *macropus eugenii*	10.7	4.5	173
14	wallaby	12.4	3.5	171
15	mallard, *anas platyrhynchos*	6.00	2.5	156
16	mallard	7.75	3.4	423
17	dog, *canis familiaris*	32	25	330
18	dog	40.0	20	347
19	dog	35.4	30	373
20	dog	51.5	23	387
21	leopard, *panthera pardus*	124	87.5	522
22	leopard	60	27	585
23	human, *homo sapiens*	142	58	580
24	human	128	93	592
25	human	158	62	718
26	human	164	65	832
27	cow, *bos primigenius taurus*	311	420	2230
28	cow	432	400	4153
29	harp seal, *phoca groenlandica*	321	145	4150
30	pilot whale, *globicephala melaena*	3155	878	20800
31	pilot whale	5621	1652	25700



(Fig.5) The figure shows a significant correlation between number of glomeruli and body weight among mammals (r = 0.97). Numbers refer to Table 2. Mammals (excluding human beings) are indicated by black dots, human beings by crosses and non-mammals by circles.

careful than of the sampled section too many glomeruli will be counted.

The efficiency can be improved by using both sections of each section pair as a sampled section, and the other one as the look-up section. Of course no glomerulus can be present in one section and absent in the other in both cases. As the estimates from the fractionator are unbiased, they will vary around the true value. How much they vary depends on the homogeneity of the particle density, the size variation of sampled pieces and the number of pieces actually counted (Gundersen, 1986). We employed systematic sampling (Gundersen and Jensen, 1987) and made sampling units of roughly equal size on each sampling step to improve efficiency. Moreover, our sampling design describes a 'smooth' fractionator, what is in agreement with Ogbuihi and Cruz-Orive (1990).

It is still a question of debate, how to estimate the efficiency of the fractionator. The 'splitting' design of Gundersen (1986) combined with eq. 17 from Cruz-Orive (1990) apparently is the most appropriate method. Using this method, our estimates of CE show, that the observed CE in our systematic sampling design is less than one could expect it to be under independence and uniformity, when counting 135 glomeruli per kidney as a mean. The efficiency of the fractionator in glomerular counting is remarkable.

The estimate of the glomerular volume on the contrary could be biased by tissue shrinkage, truncation and overprojection (Cruz-Orive, 1983). These biases are, however, much smaller when tissue is embedded in plastic rather than in paraffin (Helander, 1983). A study in our laboratory showed no shrinkage due to dehydration, embedding, sectioning and staining procedures using JB-4 plastic sections (Schmitz et al., 1990) Also, the relation between section thickness and glomerular diameter is small, which minimizes the effect of overprojection and truncation.

We find a strong positive correlation between glomerular number and BW among several animal species. The correlation between BW and KW is also highly significant. This latter correlation agrees reasonably well with Brody's (1945) observed in more than 100 mature mammals in a wide range.

The observed correlation between glomerular number and BW confirms the findings of Vimtrup (1928) in rats, cats, dogs and human beings. Kunkel (1930) found a relationship between glomerular number and body surface area, but not between glomerular number and KW. Rytand (1938) also investigated glomerular number in four different animals (from mouse to elephant). The elephant had 600 times as many glomeruli compared to the mouse. Smith (1951) has mixed the results from Kunkel and Rytand and gains a significant correlation between number of glomeruli and BW with a slope of 0.57. This value differs significantly ($2p < 0.01$) from the value obtained by us. Maybe, this difference is due to the fact that the results of Smith are based on two different maceration techniques: Kunkel used injection of glomeruli; Rytand did not — see the discussion of these and other techniques in Bendtsen and Nyengaard (1989).

The values for glomerular number for the same species is about 50% higher for the above mentioned authors compared to our results. But they all used biased methods as described in the introduction.

The number of glomeruli compared to BW does not seem to differ between mammals and non-mammals (except turtle, because a big part of its body weight is the metabolically inert and heavy shield — see Fig.5).

Apparently aquatic animals (n° 1, 10, 11, 12, 15, 16, 29, 30, 31 excluding the turtle) have more glomeruli per BW compared to terrestrial animals: The glomerular numbers for these animals are more than hundred per cent above the regression line of all the terrestrial animals. However, the slope of glomeruli in relation to BW is identical. Maybe the water and electrolyte load made a more active renal filtration a survival advantage in any aquatic environment.

Within a species — e.g. humans — the number of glomeruli is not significantly related to BSA. Instead different load of metabolic waste products is regulated by changes of glomerular size (Nyengaard and Bendtsen, submitted). *Among* species, the glomerular size is relatively invariant. Therefore the larger load of metabolic waste products in larger animals is secreted by means of more glomeruli rather than larger glomeruli. The number of glomeruli is genetically determined, while the size of glomeruli, within a species, is determined by the environment.

Obviously the size of an *effective* nephron is limited, because one super-nephron could not work. Actually, Sperber (1944) was the first to point out that the length of the tubules in all mammals approaches a limit. The functional efficiency of the kidney depends on the relations of its various dimensions, especially the general relations of such processes as fluid flow of both urine and blood and either active or passive transport to the magnitude of surfaces (glomerular and luminal) and the thickness (i.e. permeability) of membranes. All these relations would be so exaggerated and distorted in one super-nephron as to make renal activity impossible. In a similar manner this inefficiency of scaling of size imposes limits on the adaptive potential (both functional and structural) of nephronic activity and so determines whether the kidney can provide adequate survival value.

The correlation line of KW versus BW among mammals increases faster than both number of glomeruli and metabolism versus BW ($2p << 0.01$ for the comparison in both cases). The slope of KW versus BW does not differ significantly from the results of Smith (1951). Apparently, the size or volume of tubules increases relatively more compared to metabolism. Tubular volume (TV) may roughly be estimated as (kidney volume)/(number of glomeruli). The slope of TV versus BW is 0.24 among mammals. This is equal to the difference between the two slopes of KW and number of glomeruli versus BW (0.93 and 0.67 respectively).

We have simply measured the average proximal tubular diameters in kidneys from two humans, two whales and a mouse by direct application of a ruler on the projected sections. The diameter of whale tubuli differed by a factor of two from the mouse, while the human tubular diameter was roughly equal to the whales. Apparently, the increase of kidney volume caused by increase of tubular volume may mainly be derived from an increase in tubular length rather than tubular diameter.

The BW of the pilot whale (n° 31) is 40600 times larger than the BW of the mouse. The slope of metabolic rate versus BW is 0.75 (Schmidt-Nielsen, 1984,). Then the metabolism of the whale is $40600^{0.75} \approx 2900$ times larger compared to the mouse. The number of glomeruli is 3000 times larger in the whale compared to the mouse. The filtration surface area per glomerulus is probably not much larger in the whale compared to the mouse: The volume of the whale glomerulus is about 7 times larger

than the mouse glomerulus, but inspection at light microscopical level of the surface density indicates that it is much greater in the latter compared to the former. Seemingly, changes in the glomerular filtration capacity corresponds nicely to changes in metabolism, but evidently quantitative studies of the surface area of the filtration barrier at electron microscopic level are necessary before any firm conclusion can be drawn.

CONCLUSION

The estimation of glomerular number illustrates the applicability of the fractionator principle. The perspective is, that it is now simple and easy to make precise estimates of glomerular numbers in normal and diseased kidneys of any size. The larger load of metabolic waste products in larger animals is secreted by more glomeruli rather than larger glomeruli.

ACKNOWLEDGEMENTS

The skillful technical assistance of Mrs. A Larsen, Ms. A M Nielsen and L Nielsen is thankfully acknowledged. We sincerely thank D. Bloch, Museum of Natural History, Tórshavn, Faroe Islands for the donation of the pilot whale kidneys, H. Gesser, Institute of Marine Biology, Aarhus University for the donation of the turtle, Museum of Natural History, Aarhus University for the leopard kidney and N. Wreford, Department of Anatomy, Clayton, Australia for the donation of the wallabies. The help and encouragement from HJG. Gundersen is thankfully acknowledged.

REFERENCES

Arataki M. Experimental researches on the compensatory enlargement of the surviving kidney after hydronephrosis (albino rat). Am J Anat 1926a; *36*: 71-72.

Arataki M. On the postnatal growth of the kidney, with special reference to the number and size of the glomeruli (albino rat). Am J Anat 1926b; *36:* 399-436.

Baddeley AJ, Gundersen HJG, Cruz-Orive LM. Estimation of surface area from vertical sections. J Microsc 1986; *142:* 259-276.

Bendtsen TF, Nyengaard JR. Unbiased estimation of particle number using sections — a historical perspective with special reference to the stereology of glomeruli. J Microsc 1989; *153:* 93-102.

Brody S. Linear growth, form and function. In: Brody S, ed. Bioenergetics and growth. New York: Reinhold, 1945: 589.

Cruz-Orive LM. Distribution-free estimation of sphere size distributions from slabs showing overprojection and truncation, with a review of previous methods. J Microsc 1983; *131:* 265-290.

Cruz-Orive LM. Empirical variance of a fractionator estimate. J Microsc 1990; *160:* 89-95.

Cruz-Orive LM, Hunziker EB. Stereology for anisotropic cells: application to growth cartilage. J Microsc 1986; *120:* 47-80.

Damadian RV, Shwayri E, Bricker NS. On the existence of non-urine forming nephrons in the diseased kidney of the dog. J Lab Clin Med 1965; *65:* 26-39.

Eisenbrandt DL, Phemister RD. Counting renal corpuscles in tissue sections. Virchows Arch B Cell Pathol 1980; *32:* 177-184.

Elias H, Hennig A. Stereology of the human renal glomerulus. In: Weibel ER, Elias H, eds. Quantitative methods in morphology. Berlin: Springer-Verlag, 1967: 130-164.

Gundersen HJG. Notes on the estimation of the numerical density of arbitrary profiles: the edge effect. J Microsc 1977; *111:* 219-223.

Gundersen HJG. Stereology of arbitrary particles: A review of unbiased number and size estimators and the presentation of some new ones. J Microsc 1986; *143:* 3-45.

Gundersen HJG, Bendtsen TF, Korbo L, Marcussen N, Møller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sørensen FB, Vesterby A, West MJ. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 1988; *96:* 379-394.

Gundersen HJG, Jensen EB. The efficiency of systematic sampling in stereology and its prediction. J Microsc 1987; *147:* 229-263.

Helander KG. Thickness variations within individual paraffin and glycol methacrylate sections. J Microsc 1983; *132:* 223-227.

Heptinstall RH. Anatomy. In: Heptinstall RH, ed. Pathology of the kidney. Boston: Little, Brown and Company, 1983: 1-61.

Jackson CM, Shiels M. Compensatory hypertrophy of the kidney during various periods after unilateral nephrectomy in very young albino rats. Anat Rec 1927; *36:* 221-237.

Kittelson JA. The postnatal growth of the kidney of the albino rat, with observation of an adult human kidney. Anat Rec 1917; *13:* 385-397.

Kunkel PA. The number and size of the glomeruli in the kidney of several mammals. Bull Johns Hopkins Hosp 1930; *47:* 285-291.

Larsson L, Aperia A, Wilton P. Effect of normal development on compensatory renal growth. Kid Int 1980; *18:* 29-35.

McLachlan MSF, Gaunt A, Fulker MJ, Anderson CK. Estimation of glomerular size and number from radiographs of the kidney. Brit J Radiol 1976; *49:* 831-835.

Moore RA. The total number of glomeruli in the normal human kidney. Anat Rec 1931; *48:* 153-168.

Moritz AR, Hayman JM. The disappearance of glomeruli in chronic kidney disease. Am J Pathol 1934; *80:* 505-518.

Nelson BT. The number of glomeruli in the kidney of the adult rabbit. Anat Rec 1922; *23:* 355-361.

Nyengaard JR, Bendtsen TF. The number and size of glomeruli, kidney weight and body surface area in normal human beings (submitted).

Ogbuihi S, Cruz-Orive LM. Estimating valve number with the fractionator. J Microsc 1990; *158:* 19-30.

Rytand DA. The number and size of mammalian glomeruli as related to kidney and to body weight, with methods for their enumeration and measurement. Am J Anat 1938; *62:* 507-520.

Saltikov SA. The determination of the size distribution of particles in an opaque material from a measurement of the size distribution of their sections. In: Elias H, ed. Stereology. New York: Springer-Verlag, 1967: 163-174.

Schmidt-Nielsen K. Vertebrate excretion. In: Schmidt-Nielsen K, ed. Animal physiology. London: Cambridge University Press, 1975: 458-461.

Schmidt-Nielsen K. Metabolic rate and body size. In: Schmidt-Nielsen K, ed. Why is animal size so important? London: Cambridge University Press, 1984: 56-75.

Schmitz A, Nyengaard JR, Bendtsen TF. Glomerular volume in type 2 (non-insulin-dependent) diabetes estimated by a direct and unbiased stereological method. Lab Invest 1990; *62*: 108-113.

Schweigger-Seidel F. Die Anzahl der Harnkanälchen. Längen- und Breitenverhältnisse derselben. In: F. Schweigger-Seidel, ed. Die Nieren des Menschen und der Säugethiere in ihren feineren baue. Halle: Buch des Waisenhauses, 1865: 48-50.

Smith HW. Comparative physiology of the kidney. In: Smith HW, ed. The kidney. Structure and function in health and disease. New York: Oxford University Press, 1951: 562-575.

Sperber I. Studies on the mammalian kidney. Zool. Bid. Från Uppsala, 1944; *22:* 406-411.

Sterio DC. The unbiased estimation of number and sizes of arbitrary particles using the disector. J Microsc 1984; *143:* 127-136.

Vimtrup BJ. On the number, shape, structure and surface area of the glomeruli in the kidneys of man and mammals. Am J Anat 1928; *41:* 123-151.

Weibel ER, Gomez DM. A principle for counting tissue structures on random sections. J Appl Phys 1962; *17:* 343.

Wicksell SD. The corpuscle problem. A mathematical stydy of a biometric problem. Biometrika 1925; *17:* 84-99.