Virchows Arch. A Path. Anat. and Histol. 369, 283—291 (1976)

Measuring Glomerular Diameters in Tissue Sections

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Received November 5, 1975

Summary. In some kidney diseases it is important to know the diameters of the glomeruli. If only tissue sections are available, glomeruli are cut at different levels, and direct estimation of the real diameter is impossible. A variant of Hennig's method is presented which allows calculation of the real glomerular diameters, and frequency distribution of the diameters in the glomerular population. The accuracy and reliability of the method are demonstrated.

Key words: Glomeruli — Tissue sections — Morphometry.

Some kidney diseases are characterized by enlargement of the glomeruli. In order to substantiate the diagnosis it is important to be able to estimate the glomerular diameter or radius in different types of nephropathies. Some authors only measure diameters or surfaces in tissue sections, without taking into account the fact that glomeruli are cut at variable distances from the equatorial plane.

Several authors demonstrated that all glomeruli measured in suspension (Fetterman, 1965), or in thick sections (Abrams, 1963; Elias, 1967) are a population, with normal distribution of their diameters, characterized by a mean and a standard deviation. Some mathematical problems arise when one is interested in the real diameter of the population of glomeruli present in a kidney biopsy.

Methods have been published for the calculation of the real diameter of spherical structures from measurements on random section planes. These methods are thoroughly reviewed in the publications by Elias and Hennig (1967) and by Underwood (1970). All these methods are based on large numbers of measurable particles, and need a complicated computation, or tiresome graphical constructions.

A modification of the method by Hennig (Elias, 1967) is presented, which gives good estimations of the frequency distribution and mean diameters of the glomeruli.

Materials and Methods

Kidney tissue was fixed in Bouin's fixative, or in buffered formalin, and processed through paraffin. Tissue sections were cut 2–5 micron thick and stained with a PAS or a methenamine silver method.

Surface areas of all glomerular profiles (including the capsular basement membrane) in the slide were measured using a point counting method (Hennig, 1963). For this purpose a calibrated grid (400 points) incorporated in the eyepiece of the microscope was used. A magnification was chosen, so that the average number of points falling on a glomerulus was not less than 25. From the surface areas measured in the slides, the radius was calculated

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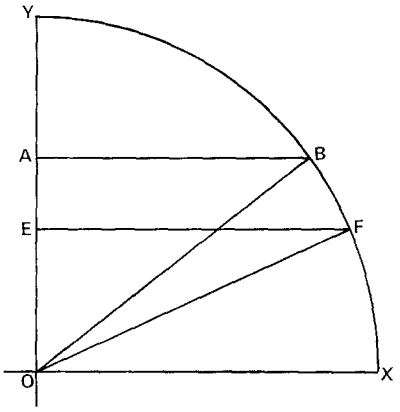

Fig. 1. Geometry involved in the probability calculations for Table 1. $OA = a$; $OE = a+1$; $AB = d$; $EF = d+1$; $OY = OX = OB = OF = D$; $D^2 = a^2 + d^2 = (a+1)^2 + (d+1)^2$.

assuming that the glomerulus was a sphere. A frequency distribution table of the obtained radii was constructed with fixed class intervals of 10 micron for kidneys with larger glomeruli and of 5 micron for the kidneys with smaller glomeruli (e.g. children or animals).

The method presented, as the method of Hennig, is based on a few principles.

1. The population of glomeruli, in which the diameter varies continuously, is considered to be composed of a number of groups with a discrete diameter interval: e.g. a population with a variation from 50 to 100 micron is considered to be composed of a group of glomeruli with a diameter of 100, a group with a diameter of 90 etc (Elias, 1967).

2. The glomerular profiles in the tissue section, belonging to the largest class of profiles e. g. 90–100 micron, are assumed to be cut near the center of glomeruli with the upper class limit (100 micron) (Elias, 1967).

3. In sectioning glomeruli, they may be cut at any distance (a) from the center, the radius of the cut profile (d) being smaller, the greater the distance from the center (Fig. 1). From the general principles of stereometry it is known that the chance to hit an object is propertionate to its volume or, that the chance to find a profile radius larger than d, is relative to the distance a. In Fig. 1 it can be seen that

$$a = \sqrt{D^2 - d^2}. \tag{1}$$

Likewise, the chance of finding a profile radius larger than d, but smaller than $d+1$ is proportionate to the distance $AE = a - (a+1)$. This distance can be calculated as

$$a - (a+1) = \sqrt{D^2 - d^2} - \sqrt{D^2 - (d+1)^2}. \tag{2}$$

If a glomerulus is cut, the probability it is hit at a level between $a - (a+1)$ equals

$$p = \frac{a - (a+1)}{D} \tag{3}$$

i. e. the relative thickness of the slice $a - (a+1)$. Now combining (2) and (3)

$$p = \frac{a - (a+1)}{D} = \frac{\sqrt{D^2 - d^2} - \sqrt{D^2 - (d+1)^2}}{D} \tag{4}$$

is obtained. This formula was used to calculate Table 1.

p: probability of finding a section with a radius falling in the class d to $(d+1)$.

D: real radius of the glomerulus.

The example illustrated in Table 2, is from a slide containing 61 glomerular profiles. Examination of the glomerular population revealed 10 profiles with a radius of 90–100 mi-

Table 1. Probability matrix. Each column (D) gives the probabilities of finding a sectional radius of a given range (d), when cutting through spheres with a radius D. The sum of probabilities per column equals 1.

d	D																		
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
0– 1	.1340	.0572	.0318	.0202	.0140	.0103	.0078	.0062	.0050	.0041	.0035	.0030	.0026	.0022	.0020	.0017	.0015	.0014	.0013
1– 2	.8660	.1975	.1022	.0633	.0432	.0314	.0239	.0188	.0152	.0125	.0105	.0089	.0077	.0067	.0059	.0052	.0046	.0042	.0038
2– 3		.7454	.2046	.1165	.0768	.0548	.0412	.0322	.0259	.0212	.0178	.0151	.0130	.0113	.0099	.0082	.0078	.0070	.0063
3– 4			.6614	.2000	.1207	.0829	.0610	.0470	.0374	.0306	.0254	.0215	.0185	.0160	.0140	.0124	.0110	.0099	.0089
4– 5				.6000	.1926	.1208	.0854	.0643	.0505	.0408	.0337	.0284	.0234	.0210	.0183	.0162	.0144	.0128	.0116
5– 6					.5528	.1848	.1192	.0861	.0660	.0526	.0430	.0360	.0305	.0263	.0229	.0201	.0178	.0159	.0143
6– 7						.5151	.1773	.1168	.0859	.0668	.0538	.0445	.0375	.0321	.0278	.0244	.0215	.0192	.0172
7– 8							.4841	.1704	.1141	.0850	.0669	.0544	.0454	.0385	.0332	.0289	.0255	.0226	.0202
8– 9								.4581	.1641	.1114	.0839	.0666	.0547	.0459	.0392	.0340	.0298	.0263	.0235
9–10									.4359	.1584	.1087	.0826	.0661	.0546	.0462	.0397	.0345	.0304	.0270
10–11										.4166	.1531	.1060	.0813	.0655	.0544	.0462	.0399	.0349	.0309
11–12											.3997	.1483	.1035	.0799	.0647	.0541	.0462	.0401	.0352
12–13												.3846	.1439	.1011	.0785	.0639	.0537	.0460	.0401
13–14													.3712	.1399	.0988	.0771	.0631	.0532	.0548
14–15														.3590	.1361	.0967	.0758	.0623	.0527
15–16															.3480	.1327	.0946	.0745	.0614
16–17																.3379	.1295	.0927	.0732
17–18																	.3287	.1264	.0909
18–19																		.3201	.1236
19–20																			.3122

Table 2. Sample calculations from a slide containing 61 glomerular profiles

Radius class (micron) of measured profiles			Real radius, calculated							
			$D=100$		$D=90$		$D=80$		$D=70$	
		A	P	R	P	R	P	R	P	R
d_1	90–100	10	10[a]	0	—					
d_2	80–90	16	4[b]	12	12	0	—			
d_3	70–80	14	3[c]	11	4	7	7	0	—	
d_4	60–70	10	2	8	3	5	3	2	2	0
d_5	50–60	7	2	5	2	3	2	1	1	0
d_6	40–50	3	1	2	2	0	—			
d_7	30–40	1	1	0	—					
Total		61	23		23		12		3	

A = number of glomerular profiles actually observed in each class d. P = number of glomerular profiles of each class d predicted to belong to each class D. R = remainder $R=A-P$, total observed (A) for that class d less those predicted (P) to belong to D.
Mean diameter: 90.8 μ. Standard deviation: 8.7 μ.
[a] 10 = predicted total $22.9 \times 0.4359 = 10$ (see text and Table 1).
[b] 4 = Predicted total $22.9 \times 0.1641 = 3.53 \sim 4$.
[c] 3 = predicted total $22.9 \times 0.1141 = 2.61 \sim 3$ etc.

cron, the largest class present in the specimen. By application of principle no. 2 we assume that all these are sections of glomeruli with a real radius of 100 micron.

In Table 1 we can find that the probability of finding 10 profiles with a radius of 90–100 micron cut from glomeruli with a real radius of 100 micron equals 0.4359 (Table 1: $D=10$, $d=9$–10), or that if we find 10 find profiles with this radius, total number of glomeruli with a radius of 100 micron should be 10: $0.4359 = 22.9 \sim 23$.

From this total, we should also find profiles in the lower ranges, the probabilities of which are found in Table 1, in the same column ($D=10$).

d_1 90–100: $22.9 \times 0.4359 = 10$

d_2 80–90: $22.9 \times 0.1641 = 3.53$

d_3 70–80: $22.9 \times 0.1141 = 2.61$ etc.

For the convenience of calculation, all figures are rounded off to the nearest whole number, which does not substantially influence the final result.

The predicted number of smaller profiles (P in Table 2) is substracted from the observed number (A), and the remainder (R) is treated in the same way in column $D=90$. Now the 12 profiles of class d_2 80–90 micron are the largest ones available. Considering them to originate from glomeruli with a raius of 90 (principle no. 2), there should be a total of 12: 0.4581 = 26.2 (0.4581: Table 1; $D=9$, $d=8$–9). From this total, we should find in class d_2 $26.2 \times 0.4581 = 12$ and in d_3 $26.2 \times 0.1704 = 4.46$ etc.

It should be noticed that the estimated number of glomeruli for $D=90$ should be $12 : 0.4581 = 26.2$, and that this number is not met in the total (23) of this $D=90$ column. Indeed for d_7 we should find $26.2 \times 0.0470 = 1.23$, $d_8 = 0.84$, $d_9 = 0.49$ etc. For the convenience of calculation in the example no negative are accepted.

The discrepancy derives from the fact that too few small glomerular profiles are identified, since grazing sections through the imperfect spheres are not identified as glomeruli (Elias, 1967).

Once the total number of observed profiles is exhausted in the calculation, the original population of glomeruli through which the sections were made can be reconstructed from the totals of each D column and statistical procedures can be applied.

Results

1. Accuracy of the Method

The accuracy was tested in a model. Circles of known diameters were drawn, and sections made at regular or at "at random" positions. The results are found in Table 3. There is very good agreement between the mean of the models and the mean calculated with this method. The differences are smaller than 3%, even with few sections (mod. 2A). In the models (4 and 5), with a small diameter, the deviation is more important, because in the lower range of Table 1, there are fewer class intervals, which makes the estimate less accurate. By multiplying the measured diameters with a constant, more classes can be used (the left of Table 1), and the deviation of the calculated mean diameter from the real mean diameter is greatly reduced (Table 3, mod. 4b and 5b).

If one compares the original frequency distribution and the calculated one (Fig. 2), a few remarks can be made. The original frequency distribution chart is constructed on the class middle. In the calculated frequency distribution, the bars are constructed at the upper class limit, because of the assumption, that all sectional diameters in one class are derived from circles of the upper class limit (see methods, principle no. 2).

The calculated frequency distribution corresponds remarkably well to the original one, expect in model one and in model two, in which the circles were all of the same size. In model one, the circle diameter was situated in the class middle, so that principle no. 2 (see methods) was not valid. In model two the circle diameters were situated on a class limit, and principle no. 2 was valid. Approximatively 73% of the circles are correctly estimated, the others being under- or overestimated by only one class. In the other models with a clear dispersion of the diameters, the calculated frequencies fit very well with the original.

Table 3. Accuracy of calculating mean diameter

	Number of circles	Number of sections	Mean diameter	Calculated mean diameter	Deviation[a]
Model 1	1	115	114	115.6	+1.38
Model 2	5	400	80	81.4	+1.70
Model 2A	5	37	80	78.2	−2.24
Model 3	50	50	78.6	78.4	−0.25
Model 4	54	54	44.0	45.7 44.3[b]	+3.89 +0.67[b]
Model 5	21	21	39.2	41.4 38.1[b]	+5.38 −2.81[b]

[a] $\left(\frac{\text{calculated mean diameter}}{\text{mean diameter}} \times 100\right) - 100$

[b] Calculated with class interval = 5, and computed from Table 1, with values of diameter multiplied by two.

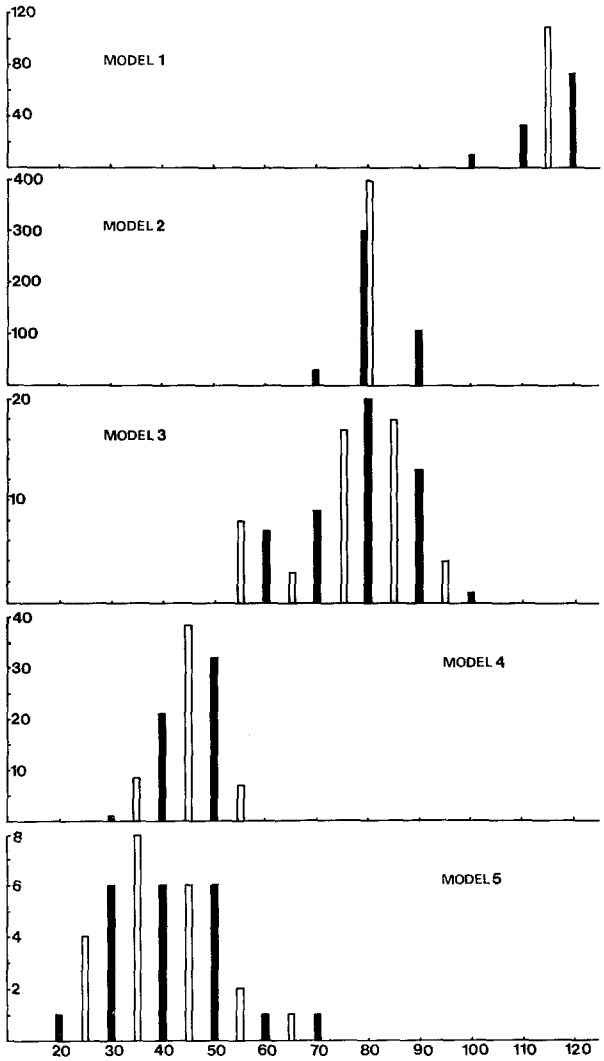

Fig. 2. Accuracy in reconstructing the population of circles, used in Table 3. Open bars are original circles, full bars are circles calculated with present method. Abcissa: diameters in millimeters; ordinate: number of sections

2. Reliability

The reliability can be defined as a measure for the accuracy in the performance on actual biopsy material. It is of course not possible to know the exact dimensions of glomeruli, without measuring them. Different methods can be tried. One consists in calculating glomerular diameters on a whole biopsy, and comparing these values with the calculations made using a sample one half of the glomeruli. This could give an idea of how reliable the method is in estimating the glomerular diameter with relatively small numbers of measurable glomeruli. Re-

Table 4A. Reliability of estimation of radius, same slide two sample measurements.

Material		Sex	Total group		Sample 1[a]		Sample 2[a]	
			n	mean	*n*	mean	*n*	mean
Normal rat	197 g	♀	50	54.0	25	55.8	25	53.8
Normal rat	210 g	♀	50	51.4	25	52.0	25	51.2
Normal dog	15 kg	♂	50	58.1	25	58.2	25	57.4
Normal human	53 y	♂	50	78.2	25	77.6	25	76.8
Human, compens. hypertr.	68 y	♂	50	94.2	25	97.6	25	88.8
Human, controlat. tromb. ren. art., hypertension	29 y	♂	62	91.1	31	85.8	31	95.2

Table 4B. Reliability of estimation of radius, different slides

Material: Human		Sex	Slide I		Slide II	
			n	mean	*n*	mean
Hematuria, no lesions	10 y	♂	27	66.3	26	64.2
Hematuria, no lesions	15 y	♂	27	83.5	25	80.0
Focal glomerulonephritis	13 y	♀	15	70.6	17	71.9
Compensatory hypertrophy	43 y	♂	9	97.9	9	90.0
Compensatory hypertrophy	4 y	♀	21	79.5	10	79.0
Compensatory hypertrophy	37 y	♂	14	131.8	22	139.4

[a] Consecutively measured glomerular profiles were numbered, and the 2nd, 4th, 6th etc. glomeruli (sample 1), compared with the 1st, 3rd, 5th etc. (sample 2).

sults of this test can be found in Table 4A. In this it can be seen that the differences are small.

If two different sections from opposite ends of the tissue block are measured and calculated, also only small differences are noted (Table 4B).

3. Comparison with Other Measurements (Table 5)

From a kidney specimen of a normal $1^6/_{12}$ years old boy, fifty juxtamedullary and fifty subcapsular glomeruli profiles were measured. The surface areas were normally distributed, but had a large standard deviation. The radii calculated from these surface areas had a skewed distribution ($p < 0.01$), with a smaller standard deviation. Using the present method, the distribution again was normal, with a narrow standard deviation. The influence of these computations on Student's *t*-test, in the evaluation of the differences between the mean radii of juxtamedullary and subcapsular glomeruli can be seen (Table 5).

Discussion

The method described offers some advantages over other previously published methods, and harbours some of the theoretical and practical inconveniences of morphometrical techniques.

The major advantage of the presented method is the simplicity of the step by step calculations, using the coefficients of table one. Once familiar with the

Table 5. Comparison between surface measurement, radius measurement and radius computation

	Juxtamedullary $n=50$			Subcapsular $n=50$			t[d]	P
	mean	SD	D[c]	mean	SD	D[c]		
Surface area $\mu^2 \times 10^3$	8.05	2.77	34.4	6.51	2.32	35.6	3.01	<0.005
R measured μ[a]	49.73	9.54	19.2	44.72	8.53	19.1	2.77	<0.01
R computed μ[b]	58.50	4.97	8.5	52.70	5.55	10.53	5.50	$\ll 0.001$

Boy $1^1/_2$ years, normal kidneys.
50 glomeruli were measured within one medium power field from medulla, or from the capsule.
[a] R measured, is the radius derived from the measured surface area, assuming this being a circle.
[b] R computed, is the radius as computed with the presented method.
[c] $D = \frac{\text{SD} \times 100}{\text{mean}}$.
[d] t = student's t, comparing the juxtamedullary and subcaprular glomeruli.

method it takes only a few minutes to calculate the real diameters of the glomerular population present in the tissue section.

In our method no correction was made for shrinkage due to fixation or embedding. Sheehan and Lynch (1973) calculated this to be 21–22% from the original diameter of the tuft. It is probable that the tuft is shrunken more than the capsule of the glomerulus. Thus fixation and embedding introduce a systematic error, which can be assumed to be approximatively constant. The variability of this error is probably smaller than the resolving power of the method, and has been ignored in prior methods (Abrams, 1963; Elias, 1967).

Instead of measuring cords at right angles (Elias, 1967), we measured the surface of the section plane through the glomeruli, and calculated the radius from this surface. This results in a more reliable estimate of the radius. The accuracy of a point counting method is independent of the geometry of the object and is proportionate only to the square root of the number of points counted (Hennig, 1963), while in cord measurements the accuracy is fixed for the instrument and the magnification, and depends on the geometry of the object to be measured.

We recommend that an average of at least 25 points are counted per glomerulus, which gives a fairly accurate and reproducible result. In order to decrease the error of measurement by one half, on the average 100 points per glomerulus must be counted, which is very time consuming.

It has been shown (Abrams, 1963) that the glomerulus is significantly different from the sphere, and that it is not randomly oriented. These differences are however small, and the equatorial diameter is only about 5% larger than the polar diameter. The elimination of this geometrical aberration is not possible in biopsy specimens.

Some authors only measure surface areas in sections of glomeruli assumed to be cut centrally, or of all glomeruli. If one choses to measure the surfaces of all glomeruli seen in the section, a more or less symmetrical distribution is found with an enormous spreading (Table 5). If from these surfaces the radius or dia-

meters are computed a skewed distribution is found. The calculated "real" diameter using our method, results in a more or less gaussian distribution with narrow spreading (see Table 5).

If not all glomeruli are to be measured the difficulties of selecting proper and reproducible criteria for including or excluding glomeruli poses a problem which is circumvented in the present method.

The major drawback of applying any quantitative method to biopsy specimens is that the sample must be representative. If the range of diameters of the structures to be measured is small, as in the normal kidney (Elias, 1967), no problems are to be expected. If however, the kidney disease causes a widening of the range of glomerular diameters, or creates a new population of diseased glomeruli, then the problem of representativity becomes very important when working with small numbers of measurable glomerular profiles.

The authors wish to thank Dr. R. L. Vernier and Dr. J. D. Elema for constructive criticism in the elaboration of this paper.

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