Dimensional Interrelationships of the Proximal and Distal Tubules of the Nephrons in the Dog[1] (35692)

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The structural organization of the nephron is consistent with a progressive change in the volume and composition of glomerular filtrate as it passes down the tubule. Reabsorption of solute and water seems to begin as soon as filtrate enters the proximal convoluted tubule and continues thereafter in proportion to the load. Since the TF/P ratio of inulin remains relatively constant at any point over a wide range of GFR it has been inferred that a functional "glomerulotubular balance" arises actively from appropriate changes in reabsorption in response to alterations in filtered load. By this mechanism, which is not yet understood nor clearly defined (1), an inherent variation in nephron function is minimized. If the remarkable diversity of the nephron population evident in a two- to fourfold range in glomerular and tubular dimensions (2, 3) were coupled with an equal diversity in nephron activity, precise and predictable control of overall activity by neural and humoral mechanisms would be greatly impaired. Non-uniform distribution of the excretory load might also have deleterious effects. This potential variance appears to be modulated by anatomic as well as physiologic integration. Glomerular size has been found (2, 3) to be correlated in dog and man with volume of the attached proximal convoluted tubules, suggesting that the tubular cell mass is approximately geared to the filtered load imposed upon it. Since function of the proximal tubule, in turn, determines the volume and composition of the residual filtrate entering the distal tubule, it may be inferred that the volumes of the proximal and distal convoluted tubules are correlated in the same manner. This inference has been borne out by the study reported herewith.

Methods. Measurements of the dimensions of proximal and distal tubules were made on nephrons obtained by microdissection (2, 3) from the kidneys of three female dogs (Q, P, and E), weighing 16, 14, and 15 kg, respectively. All were in good health and renal function studies had yielded normal results shortly before sacrifice. In each instance the kidneys (weighing 28, 28, and 36 g, respectively) were removed, bisected, and fixed in 10% formalin for at least 4 weeks. In none was there any evidence of renal or urinary tract pathology on gross and microscopic examination.

Small thin wedges of tissue which included cortex and medulla were taken from one of each pair of kidneys at uniformly distributed points from the polar, equatorial, and intermediate zones. The tissue was macerated for 5 hr in concentrated hydrochloric acid at 37 ± 0.5°, washed with distilled water or isotonic saline solution, and then allowed to stand approximately 20 hr in water or saline at 4–7°. A small portion of the tissue block was then teased apart in distilled water or saline and the nephrons separated under a binocular dissecting microscope. Complete proximal and distal tubules with their attached glomeruli were isolated randomly in equal numbers from beneath the capsular surface, midcortex, and corticomedullary border. Care was taken to maintain the integrity of the distal tubule from the point of its tight attachment at the vascular pole of the glomerulus of origin to terminus in the collecting tubule. Although the fragility of the thin

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limb nearly always resulted in breakage, the tight connection between the distal tubule and the glomerulus of origin, as well as the ease of defining both distal and proximal tublues, assured accurate identification of tubules from the same nephron. The isolated nephrons were floated onto a slide in water or saline solution, and after gentle manipulation spread sufficiently to permit a camera lucida drawing, such as illustrated in Fig. 1, of each nephron. The proximal tubule was defined as that portion of the nephron extending from the glomerulus, indicated in Fig. 1 by A, to the thin portion of Henle's loop, indicated by B. The first three-quarters is convoluted and the remainder, beginning at T, is relatively straight. The distal tubule begins at the macula densa, shown at C. Approximately three-quarters (C to E) coils within the loops of the associated proximal convolutions. The second portion, the connecting tubule, is characterized by a smaller cross section and less granular cellular structure. The terminal portion of two or more connecting tubules, the so-called "arcade," ends in the collecting tubule, marked D.

Using an odometer, measurements of tubular length were made from A to B, and from C to D, for the proximal and distal lengths,

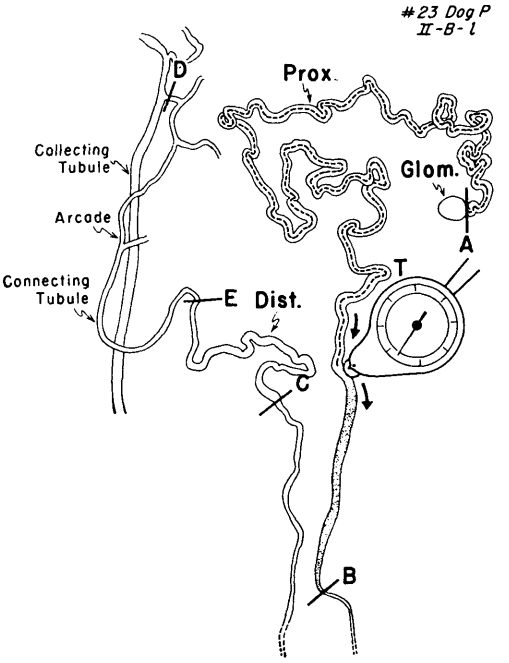

FIG. 1. Camera lucida drawing of proximal and distal tubules. The lengths of the proximal tubule, from A to B (composed of a convoluted portion and a straight terminal portion beginning at T) and the distal tubule, from C to D (composed of convolutions (C to E) and connecting tubule) and measured by an odometer shown here diagrammatically.

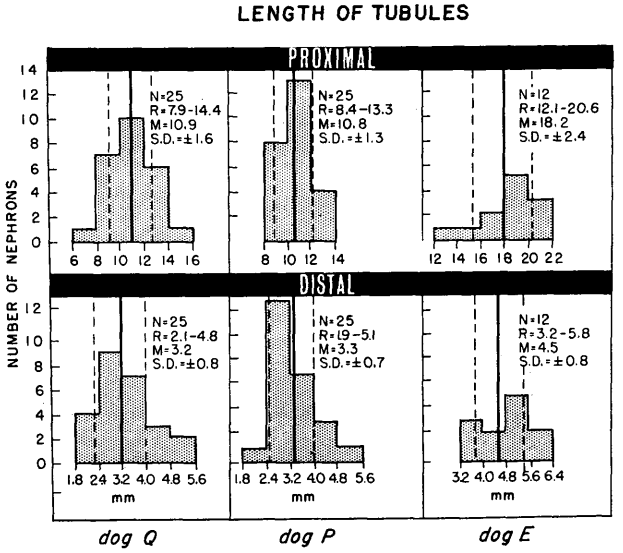

FIG. 2. Distribution of tubular lengths. (*N*, number of tubules measured; *R*, range of values; *M*, mean; and *SD*, standard deviation).

respectively. The diameters of each tubule (taken at 10 points evenly distributed along its course) were measured directly with an eye-piece micrometer at a magnification of 100. The values were averaged and the volume of each tubule (*i.e.*, A to B for proximal and C to D for distal) was computed as for a cylinder.

Results and Comment. The distribution of total proximal and distal tubular lengths (all values obtained after microdissection in distilled water only) for 25 nephrons from the kidneys of dogs Q and P, and for 12 nephrons from dog E is presented graphically in Fig. 2. They averaged 10.9 ± 1.6 (SD), 10.8 ± 1.3, and 18.2 ± 2.4 mm, respectively, for proximal lengths as compared with 3.2 ± 0.8, 3.3 ± 0.7, and 4.5 ± 0.8 mm for distal lengths. The values were distributed fairly closely about the mean, the coefficient of variation ranging from 12 to 15% for proximal lengths and from 18 to 25% for distals. The ratios between mean proximal and distal lengths were 3.41, 3.27, and 4.04 for Q, P, and E, respectively. For individual nephrons, the ratios averaged 3.43 ± 0.76, 3.49 ± 0.16, and 4.11 ± 0.67, respectively.

The values for the diameters (in distilled water) (Fig. 3) of the proximal tubules averaged 39 ± 4, 46 ± 5, and 58 ± 6 μ and the coefficient of variation ranged from 10.3 to 10.9% for the three dogs. The distal diameters were always smaller in any single nephron and averaged 32 ± 4, 36 ± 3, and 42 ± 4 μ, respectively, for the sample as a whole with the coefficient of variation ranging from 8.3 to 12.5%. The ratios between proximal and distal diameters amounted to 1.22, 1.28, and 1.38, respectively, in dogs Q, P, and E. For individual nephrons, the ratios averaged 1.24 ± 0.16, 1.32 ± 0.14, and 1.36 ± 0.07, respectively. In single nephrons the proximal tubule was always larger than the distal. It is also evident that the ratios between segments were relatively constant between dogs as well as within the nephron population of a single animal.

This same phenomenon was evident also with respect to volume. The mean proximal volumes were 13.3 ± 4.2 × 10^{-3} in Q, 18.9 ± 6.3 × 10^{-3} in P, and 48.5 ± 12.5 × 10^{-3} mm^3 in E, while the respective mean distal volumes amounted to 2.6 ± 0.9 × 10^{-3} in Q, 3.2 ± 0.7 × 10^{-3} in P, and 6.5 ± 1.7 × 10^{-3} mm^3 in E (Fig. 4). The proximal tubular cross-sectional areas were always larger than distal cross sections in individual nephrons, the proximal to distal tubular cross-sectional area ratios for Q, P, and E averaged 1.54, 1.76, and 1.84, respectively, whereas the

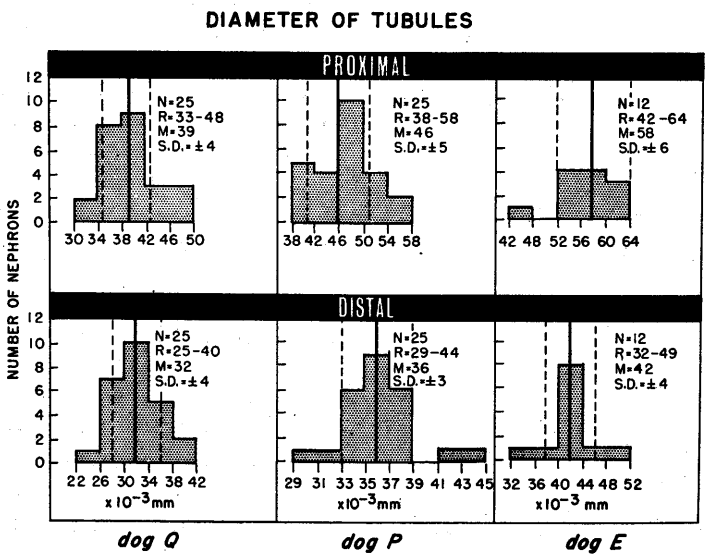

FIG. 3. Distribution of mean tubular diameters. Values are averages of 10 diameters measured at equal distances along each tubule. Abbreviations as in Fig. 2.

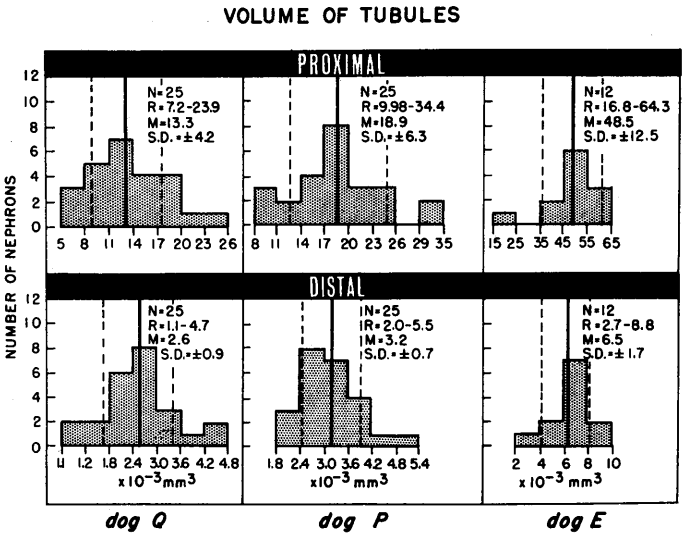

FIG. 4. Distribution of tubular volumes. Each value was computed from length and average diameter as for a cylinder. Abbreviations as in Fig. 2.

proximal to distal length ratios averaged 3.43, 3.49, and 4.11, respectively. Hence, it may be concluded that distal tubular volume differed from proximal volume largely as a function of difference in length.

The ratios between mean proximal and mean distal volumes were 5.1, 5.9, and 7.5 for Q, P, and E. For individual nephrons the proximal–distal volume ratios averaged 5.34 ± 1.32, 5.88 ± 1.46, and 7.59 ± 1.51. This agreement between values for ratios between the mean tubular volumes and for the average of individual values suggests that the volumes of the proximal and distal tubules were closely correlated despite considerable inherent dimensional variance. This inference was borne out by the correlations evident in dogs Q, P and E in Fig. 5, where ellipses, calculated to enclose approximately 70% of the values for the total volumes of distal and proximal tubules of the same nephron, have been plotted on log-log paper in order to indicate the proportional, as well as absolute, relationships. For each animal the volumes were significantly correlated: $r = 0.761$ for Q ($p<0.001$), 0.679 for P ($p<0.001$), and 0.714 for E ($p<0.01$). An even closer correlation is evident in the total pooled data than within each dog.

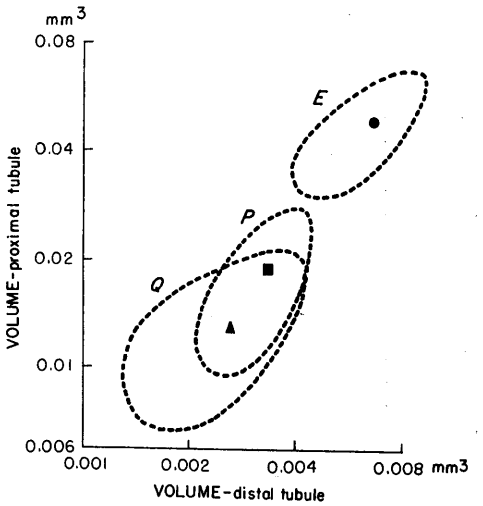

FIG. 5. Correlation between volumes of total proximal and distal tubules. All values plotted logarithmically and ellipses constructed to contain 70% of the data for each animal (see Ref. 4).

All these figures relate to the dimensions of the total proximal and distal tubules and do not necessarily reflect a similar tendency on the part of their subdivisions. The proximal convolutions comprised 70% or more of the total proximal tubule, accounting for 73.3 ± 7.4% of length in Q, 69.7 ± 7.0% in P, and 74.0 ± 5.5% in E and for 74.8 ± 9.1% of volume in Q, 71.0 ± 7.6% in P, and 77.0 ± 6.0% in E. The distal convolutions also made

up the bulk of the distal tubule. In Q, convolutions constituted 64.4 ± 13.1% of the distal tubular length; in P, 61.3 ± 12.6%; and in E, 59.0 ± 10.5%. Similarly for volume, convolutions accounted for 75.3 ± 10.9% of total distal tubular volume in Q, 70.0 ± 10.3% in P, and 69.1 ± 9.3% in E. The correlation between the volumes of the convoluted portions of the proximal and distal tubules was of border line significance but the proximal convolutions always greatly exceeded the distal convolutions in volume with ratios of 5.46 ± 1.97 for Q, 5.66 ± 1.67 for P, and 8.95 ± 2.25 for E.

It should be stressed that all these data were obtained from tubules which had been soaked in distilled water following maceration. The procedure required by microdissection is obviously associated with considerable tissue alteration owing first to the dehydration during maceration and then subsequent imbibition of water. It has been found that repeated measurements during a period of 2–3 hr in distilled water do not differ significantly, however. Hence it may be concluded that maximal swelling was achieved during 20 hr of immersion prior to dissection and continued swelling did not affect the results. All measurements were also made for dogs P and Q on equally large samples of nephrons maintained throughout in isotonic saline solution. In each instance, all dimensions were smaller than those for nephrons kept in distilled water but the relationships between proximal and distal tubules and between their subdivisions remained unchanged with values for correlations and ratios that did not differ significantly from those presented above. Although the procedure clearly makes it impossible to obtain absolute values for the dimensions of the nephron in the intact kidney, it appears to yield reasonably accurate and valid estimates of variance and correlations within the nephron population.

The data on interrelationships emerging from this study are entirely consistent with the concept of a balanced operation of the different parts of the nephron. A number of studies from this (3) and other laboratories (5) have brought forward evidence for a significant correlation between glomerular and proximal tubular dimensions in line with a functional glomerulotubular balance that assures a filtered load commensurate with the reabsorptive capacity of the proximal convoluted tubule. The proximal tubule is believed (6) to absorb approximately two-thirds to seven-eighths of the glomerular filtrate so that the loop of Henle and the distal tubule receive only one-eighth to one-third of the load initially imposed by filtration. It is interesting, therefore, to note that proximal tubular volume is 5.34–7.59 times that of the distal. The significant correlation between proximal and distal tubular volumes of the same nephron strongly suggests an intranephronic dimensional balance that minimizes functional heterogeneity and assures comparable urine formation by a diversity of nephrons. Further work is necessary to define this conclusion in terms of function. In addition, the possibility of regional differentiation of nephron activity serving the same end must be sought. A tendency was observed for superficial nephrons to be larger than those at lower cortical levels as reported in earlier studies of dog and man (2, 3). Much more data will be necessary, however, to determine if glomerulotubular and intersegmental correlations are complemented by a balanced organization of the nephron population geared to circulatory and collecting duct capacities.

Summary. Measurements of the dimensions of proximal and distal tubules were made on nephrons obtained by microdissection from the kidneys of three female dogs. In any nephron, the length, average cross section, and volume (as for a cylinder) of the proximal tubule from its attachment to the glomerulus to the thin portion of Henle's loop were always larger than the same values for the distal tubule measured from the point of attachment to the glomerular vascular pole to entry into the collecting tubule. Proximal–distal volume ratios averaged 5.34 ± 1.32 (SD), 5.88 ± 1.46, and 7.59 ± 1.51 for each of the three dogs. The proximal convolutions accounted for 74.8 ± 9.1%, 71.0 ± 7.6%, and 77.0 ± 6.0% of the total proximal volume in the same animals. The convolu-

tions bulked equally large in the distal tubule, making up 75.3 ± 10.9%, 70.0 ± 10.3%, and 69.1 ± 9.3% of the total, respectively. Proximal convolutions exceeded distal convolutions in volume, with ratios of 5.46 ± 1.97, 5.66 ± 1.67, and 8.95 ± 2.25 times, respectively. The volumes of proximal and distal tubules in the same nephron were significantly correlated in keeping with an intersegmental functional balance between residual filtered load leaving the proximal tubule and the capacity of the distal tubule to deal with it. The relative size of proximal and distal convolutions suggests that proximal reabsorbate determines the microenvironment of the distal convolutions.

1. Lassiter, W. E., Mylle, M., and Gottschalk, C. W., Amer. J. Physiol. **206**, 669 (1964).

2. Oliver, J., and MacDowell, M., J. Clin. Invest. **40**, 1093 (1961).

3. Bradley, S. E., Laragh, J. H., Wheeler, H. O., MacDowell, M., and Oliver, J., J. Clin. Invest. **40**, 1113 (1961).

4. Smith, H. W., Goldring, W., Chasis, H., Ranges, H. A., and Bradley, S. E., J. Mt. Sinai Hosp. **10**, 59 (1943).

5. Fetterman, G. H., *in* "Pathology Annual 1970" (S. C. Sommers, ed.), p. 173. Appleton-Century-Crofts, New York (1970).

6. Pitts, R. F., "Physiology of the Kidney and Body Fluids," 266 pp. Year Book Med. Pub., Chicago (1968).

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