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Drugs and the Kidney

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Contents

Section II: Renovascular Pharmacology

Section IV: Drugs Affecting Urate Transport and Metabolism

Section V: Therapy in Chronic Renal Failure

Section I: Drugs and Single Nephrons

In EDWARDS: Drugs and the Kidney
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Nephron Heterogeneity and the Evaluation of Single Nephron Function

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Contents

The technique of micropuncture of glomeruli and tubules, devised and developed by A. N. RICHARDS, J. C. WEARN, A. WALKER and their colleagues some fifty years ago, opened up the modern era in the analysis and understanding of renal physiology. Not only did it provide definitive proof of the role of the glomerulus as a filter in which an ultrafiltrate of plasma is formed by the operation of a balance of hydraulic and osmotic pressure gradients but it also permitted precise determination of segmental tubular reabsorptive and secretory functions in the cortex, provided quantitative evidence for the medullary counter-current mechanism, and made it possible to measure filtration rate directly in individual nephrons (SNGFR). Among the problems one must deal with in applying the technique are alterations in function secondary to manipulation of the kidney, trauma by direct puncture, and changes in glomerulotubular pressure gradients that may serve as important determinants of filtrate flow, but perhaps the most immediately obvious difficulty lies in the heterogeneity of nephron dimensions and functions.

Normal
Anatomic Diversity

Nephrons dissected from the kidneys of man, dog and rat have been found to vary widely in size. In man the glomerular surface was found by OLIVER and MACDOWELL [20] to average 0.257 ± SD 0.071 mm² and to range from 0.101 to 0.467 mm² and the length of proximal convoluted tubules to average 19.7 ± 2.79 mm with a similar range. Variance and range were less striking in individuals and were further modified by a statistically significant correlation between glomerular and tubular dimensions in each nephron, an anatomical glomerulotubular balance that serves presumably to adjust filtered load to tubular capacity. The same phenomenon was demonstrable in three dogs [5] where glomerular surface averaged 0.15 ± 0.024 mm² and total proximal length 24.5 ± 3.01 mm and in the rat where glomerular surface has been found [3] to average 0.0472 ± 0.008 mm² and total proximal length 8.31 ± 0.95 mm. The 3- to 4-fold spread evident in these figures implies considerable diversity in glomerular and tubular functional capacity even though glomerulotubular balance may play a moderating role. Samples of nephrons taken from different depths in the cortex and from different parts of the kidney (equatorial and polar) failed to show any significant difference in these parameters in man and dog. In the rat, however, juxtamedullary units have been found to be significantly larger than those lying below the capsule in the outermost layers of the cortex. This gradient in size from deep to superficial appears to persist throughout the life of the animal and is perhaps a phenomenon attributable to continuous growth in the rat and to the earlier formation of the juxtamedullary nephrons [1].

From these data alone it is evident that samples of urine drawn from proximal convoluted tubules on the surface of the kidney are referable in the rat strictly to a nephron subpopulation that differs dimensionally, and functionally as well in all probability, from that in the deeper cortex. Since the distal convolutions of any nephron coil within the immediate vicinity of the proximal convolutions of the same unit, the composition of tubular fluid samples from surface proximal and distal convolutions may be compared with some confidence. Samples from the loops of Henle punctured in the papilla are drawn, however, from another – juxtamedullary – subpopulation and are, therefore, more difficult to evaluate in terms of sequential change along the tubule.

Normal Functional Diversity

To test the hypothesis implicit in the foregoing comment, that size and functional capacity are correlated, it is necessary to find some means of measuring discrete nephron activities under basal conditions. The micropuncture methods have proved to be powerful tools for defining, localizing, and quantifying glomerular and tubular function but owing to the sampling difficulties, evaluation of the nephron population and its subsidiary components has not been possible. Luckily, the Hanssen technique [16] has been found to serve as a helpful approach to the problem. Precipitation of filtered sodium ferrocyanide in the glomerulus and tubular lumen as an easily visible insoluble crystalline Prussian blue makes it possible to subject fragments of kidney to hydrochloric acid maceration without loss of the markers and then to dissect out individual units in large numbers from every part of the cortex for study. When ^{14}C-labelled ferrocyanide has been administered continuously during a 10- to 12-sec period prior to rapid ligation of the renal pedicle, followed by immobilization of ferrocyanide *in situ* by snap freezing and its precipitation by acid-alcoholic ferric chloride, the Prussian blue crystals may be seen to fill only the first 50 % of the proximal tubule. Determination of the radioactivity of each unit with correction for extraluminally present ferrocyanide gives a value for the amount filtered and this divided by the mean concentration in the plasma during the period of infusion yields the single nephron glomerular filtration rate for the 10- to 12-sec interval. Pure ferrocyanide must be used that has been proved to be cleared at the same rate as inulin. This modification [11] of the Hanssen technique suffers from many of the drawbacks of micropuncture – general anesthesia, manipulation of the viscera and, in addition, administration of a substance potentially capable of lowering the blood pressure. After the ligature is loosely laid about the renal pedicle and the abdominal incision closed, each animal is given a long period of rest to permit recovery from surgery and maintenance of light anesthesia. Blood pressure has been found to change very little with this procedure. It possesses the great advantage of providing information on the statistical behavior of different parts of the nephron population. In normal rats, SNGFR in superficial (S), intermediate (I), and juxtamedullary (JM) nephrons averaged 13.7 $\pm$ SE 0.9, 13.3 $\pm$ 0.8, and 16.4 $\pm$ 0.9 nl/min/100 g body weight, respectively. The difference between S and JM SNGFR was consistent with the observed dimensional differences, with S/JM ratio for glomerular volumes

averaging 0.83 ± 0.015 in keeping with the hypothesis of dimensional-functional correlation.

The possibility that nephron subpopulations may behave disparately in renal functional adjustments has considerable theoretical appeal [6]. Modulation of the contributions of nephron groups of differing capabilities could enhance such responsivity with economy and efficiency independently of more slowly developing changes in tubular cellular transport of water and solutes. If superficial nephrons normally reabsorb less filtered sodium than juxtamedullary, for example, the need to eliminate a larger quantity of sodium during salt loading could be dealt with simply by hemodynamic adjustments that diminish filtration in JM and increase it in S nephrons. The report by HORSTER and THURAU [17] in 1968 that just such a change could be detected by the micropuncture technique in rats receiving high sodium diets over a long period gave strong support to this possibility. This finding has not been completely confirmed, however [13]. In our own experience using the modified Hanssen technique [10] no evidence of a redistribution in nephron function could be obtained. Over a 10-fold range of sodium intake and excretion (0.8–9 mEq/day) the average S/JM SNGFR ratio remained unchanged within normal limits. Excellent agreement continued to be observed between summated SNGFR and overall filtration rate (inulin clearance). The difficulty in obtaining adequate samples or in maintaining an undisturbed local circulatory setting may be responsible for the discrepancy. Whatever the explanation, the role of redistribution in normal adjustments to salt and water loading remains debatable and unsettled. It seems not unlikely, nevertheless, that subtle circulatory shifts, not easily defined by these imperfect methodologies, may prove to be much more significant than is apparent at present. Certainly the exaggerated nephron heterogeneity that develops during the course of various types of renal injuries, malformations and dysfunctions strongly suggests that redistribution of some kind has a place in normal renal physiology.

Anatomic Defects and Heterogeneity

Many studies – and in particular, those of OLIVER [19] – have clearly demonstrated a remarkable variety of glomerulotubular damage within the nephron population in glomerulonephritis, nephrosclerosis and pyelonephritis. Even when a uniformly distributed noxious agent like mercury

or uranium is involved, considerable diversity is observed despite evident involvement of every nephron. To some extent, this variability is obviously attributable to the random accidents of tubular collapse, perforation, obstruction or ischemia. Under these circumstances it is necessary to correlate anatomic and functional data for each nephron individually. Micropuncture studies [2] are yielding a rich harvest of new data and a more sophisticated understanding of the mechanisms of nephron dysfunction that demonstrate the weaknesses of such generalizations as the 'intact nephron theory' or the 'Trueta shunt'. The pathophysiology of nephron *subpopulations* remains as yet untouched. The Hanssen technique has not been employed in this area and microdissection data come slowly and with great difficulty. It is becoming increasingly evident, however, that glomerulotubular and tubular intersegmental imbalances may play an important role in the pathogenesis of abnormal urine formation. The nice coordination dimensionally between the parts of the normal nephron [9] is obviously disturbed by lesions that affect glomeruli and the tubular segments unequally and erratically. As a result, what may be viewed as 'intranephronic heterogeneity' becomes an important feature of disease. We have been especially interested in a disorder in which this appears to be a major and relatively isolated feature that appears to determine the character of renal dysfunction – hypothyroidism in the rat.

STÉPHAN *et al.* [21] and others have shown that the hypothyroid rat regularly develops a persistent polyuria and natriuresis that may be fatal if an adequate supply of water and salt is not made available. The syndrome has been attributed [14, 18] to a defect in tubular cellular transport because filtration is diminished and not increased, while proximal tubular fluid-to-plasma inulin concentration (TF/P) ratios determined by micropuncture at the end of the proximal tubule are reduced by 27 %. Recent measurements [3] of the dimensions of glomeruli and tubules from hypothyroid rat kidneys indicate, moreover, that a glomerulotubular imbalance arises from continued growth of imperfectly functioning glomeruli in association with growth arrest and atrophy of proximal and distal tubules, (fig. 1).

Since tubular fluid samples drawn from the end of the shorter-than-normal proximal convolutions (5.96 ± 1.36 mm vs. control 8.68 ± 1.16 mm; $p<0.001$) have travelled down a tubule not much more than two-thirds the normal length, a reduction in TF/P ratio would be expected even if reabsorptive activity were normal. It is possible, therefore, that the dimensional defect is sufficient alone to account for excess natriuresis.

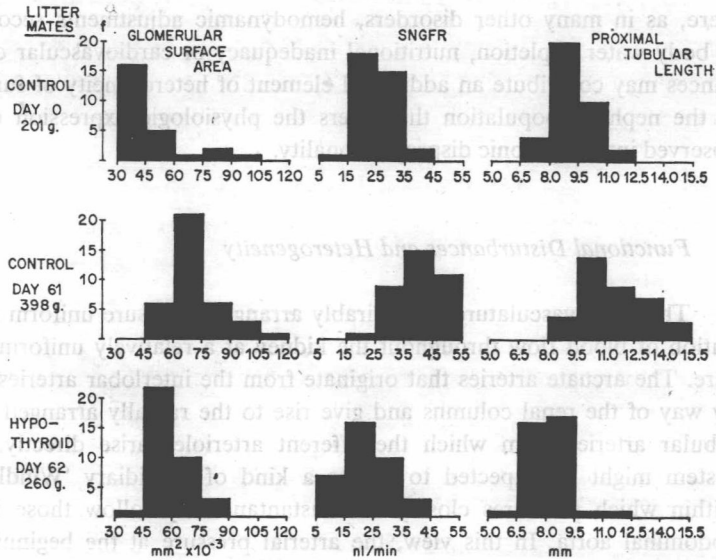

Fig. 1. Distribution of glomerular surface areas, single nephron glomerular filtration rates (SNGFR), and proximal tubular lengths in normal and hypothyroid rats. On day zero, one of three litter mates weighing 191, 201, and 207 g was given ^{131}I intraperitoneally and one was subject to study immediately. The data for the first control animal on day zero are shown in the upper horizontal panel. After 2 months the ^{131}I-treated rat had become hypothyroid and stunted in growth having gained only 69 g to reach a body weight of 260 g in contrast to its second normal litter mate control which had gained 191 g to reach 398 g body weight. Measurements made in these two animals are presented in the middle and lower horizontal panels. The 3-fold spread in glomerular surface area (GS) in the first control on day zero was associated with a similar dispersion in SNGFR (mean ± SD = 0.0495 ± 0.014 mm^2 vs. 24.86 ± 5.80 nl/min) and much less variance in length of the proximal convoluted tubule (PL) (9.01 ± 1.23 mm). With normal growth, all values increased above those for the first control rat – by 44.9, 58.5, and 26.4 %, respectively to 0.0717 ± 0.013 mm^2, 39.39 ± 7.49 nl/min and 11.39 ± 1.61 mm without change in variability. In the hypothyroid animal, however, GS rose only by 18.3 % while both SNGFR and PL actually decreased to 90.7 and 98.6 % of the values for the first litter mate control on day zero – (GS 0.0585 ± 0.011 mm^2; SNGFR 22.54 ± 9.63 nl/min, and PL 8.07 ± 1.17 mm). Although the dimensional parameters failed to show any change in dispersion, SNGFR varied more widely in the hypothyroid rat than in either of the two controls. In all animals glomerular and tubular dimensions were positively and significantly correlated. In each animal, measurements were made in 36 nephrons taken in equal numbers from the superficial, middle and juxtamedullary cortex. No change was observed in the usual superficial-juxtamedullary dimensional gradient.