Pediatric Kidney Disease

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Editor

Volume I

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Volume I

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I. The Kidney and the Urinary Tract: Morphology and Physiology

Section One. The Normal Kidney and Urinary Tract

1. Embryonic Development and Prenatal Maturation of the Kidney

Wallace W. McCrory

Some familiarity with the normal development of the kidney is of great importance to the pediatrician, general physician, urologist, and others who participate in the care of children with renal disease arising as a consequence of maldevelopment. An understanding of this process is a prerequisite if we are to increase our knowledge of the pathogenesis of dysplasia, polycystic disease, and congenital obstructive uropathy. Considerable new information about mammalian renal development has been accumulated from recent studies employing microdissection, histochemical techniques, and in vitro tissue-culture systems. It is the purpose of this chapter to review the basic knowledge currently available about the organogenesis and differentiation of the kidney in a manner useful to the clinician interested in childhood renal disease. It will not, however, be possible to present more than an overview of the vast amount of information available, and the reader interested in more detail should consult recent monographs dealing with this subject [20, 23, 27].

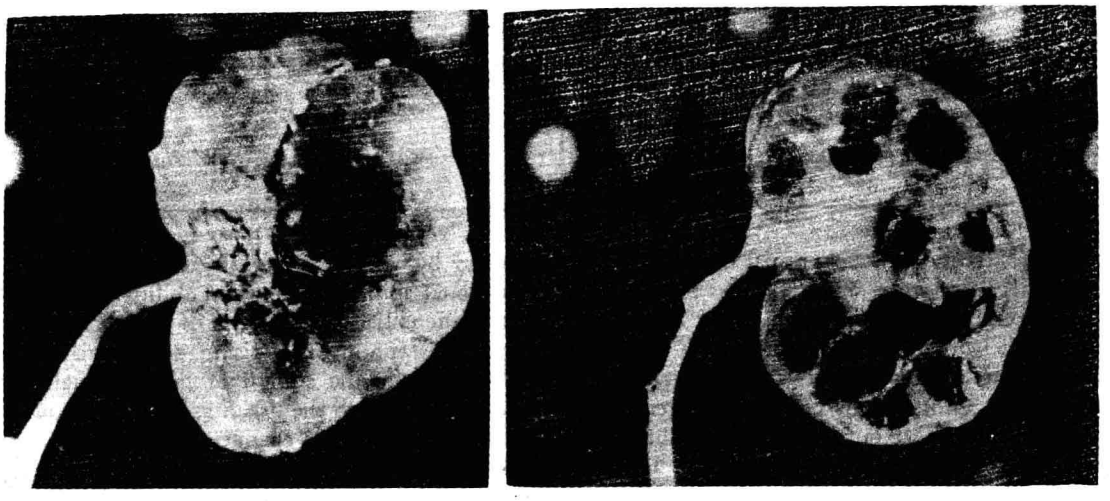
The kidney of the normal newborn, viewed in biologic terms, is a fully differentiated organ. However, in terms of gross morphology and function, significant quantitative differences between the newborn and adult kidney are clearly evident. As an example, the external surface of the newborn kidney shows prominent lobulations revealing its underlying lobular structural organization (Fig. 1-1*A*). Adaptive cortical growth obliterates fetal lobulation, and as a result the surface of the kidney of the older child or adult is completely smooth and grooveless.

Significant functional differences both in the whole organ and in single nephrons are also readily demonstrable (see Chap. 2). Morphologic and functional differences of this type have been attributed to organ maturity; findings in infants are considered as indicative of immaturity whenever they deviate quantitatively from those in the mature organ, using various standards of reference.

It is useful to distinguish between two developmental growth processes: *organogenesis,* resulting in specific tissue induction, and *maturation,* the process by which the organ acquires its unique functional abilities. Functional ability appears only after differentiation. The level of renal functioning capacity is continuously rising during maturation in response to the stimuli of greater renal work loads presented to the organ as a result of quantitative and qualitative changes in other organs and tissues accompanying normal growth and development. This type of work hypertrophy maintains equilibrium between renal load and structural-functional activity and is an integral part of the normal process underlying adaptive growth. The development of functional transport capability by specialized tissues can begin as soon as cellular differentiation has progressed sufficiently to create the subcellular (molecular) capacity for specific biochemical function and after such specialized cells have been properly organized spatially. Both the intracellular and extracellular microenvironments play a role in the processes of tissue induction and functional maturation. Once cell function begins, it can dynamically alter the composition of the microenvironment, and subsequent inductive activity may be influenced accordingly. All these processes should be viewed as operating pari passu in the early period.

In this description of renal development the term *organogenesis* will refer to the biologic processes directing induction of new tissues through the step of differentiation, and the term *maturation* will be used when referring to all the processes directing the development of functional capacity and cell growth.

Nowhere are the relations between organ structure and function illustrated more superbly than by examination of the human kidney. The kidney of man is a multilobed structure and differs from the unipapillary kidney, also found in mammals, by its organizational plan of nephrons clustered as units that make up the lobes whose collecting systems drain into a multicalicine ductal complex (Fig. 1-1*B*). This complex structure arises as a result of a developmental program set by the early branching of the ureteric bud that determines the final number and pattern of the major and minor



A B

Figure 1-1. A. Normal kidney of a newborn full-term infant, age 5 days. The individual lobes can be distinguished as lobulations visible on the surface. B. Cut section, normal kidney of a full-term newborn infant, age 5 days. Some papillae can be seen projecting into minor calyces. The fusion of three papillae into a single compound structure is visible emptying into the inferior calyceal system. The pelvocalyceal system and ureter are still attached.

calyces and renal lobes. Overall renal functional efficiency is determined not only by numbers of nephrons but also by how these units are organized to form kidneys. The dimensions and relations of the individual nephrons determine the composite functional efficiency of the kidney. Rates of filtration per single nephron and tubular fluid flow and membrane transport, active and passive, are dependent on physical factors such as surface area and membrane thickness. Individual nephron units increase in size with growth by means of both cell hyperplasia and cell hypertrophy. Renal hypertrophy provides the physiologic mechanism whereby the kidney responds with adaptive growth to requirements for increases in functional load by increasing renal mass. The total number of nephron units in man have been formed before birth; however, structural changes in the size of nephron parts occur when functional equilibrium is disturbed by either a reduction in renal mass from loss of existing nephrons by disease or an increase in work load such as occurs with increase in body size with growth. Renal compensatory growth must operate within fixed limits, however, if preservation of functional efficiency is to be maintained. If there is too great a loss of nephron mass (reduction of numbers of nephrons) the result of even maximal hypertrophy of residual nephron units will still result in a functionally inefficient kidney. Studies involving experimental reduction of renal mass have reproduced all of the phenomena of chronic renal failure. Platt and his coworkers [26] demonstrated that when five-sixths of the kidney of the adult rat was removed, the compensatory hypertrophy of the residuum could not maintain renal function adequate to support normal life. Morrison [21] demonstrated that this applies equally well to young, growing subjects. The pathophysiologic consequences of reduction in nephron numbers have been extensively studied by Bricker and associates [7]. The interrelationships between structure and function are central to the consideration of the process of development of the normal kidney.

The early stages involved in the development of the human kidney afford a dramatic illustration of the principle that "ontogeny recapitulates phylogeny." Development proceeds from a simple system of aglomerular tubules through simple glomerulonephrons to culminate in the elaboration of the final complex glomerulonephron units found in the mature kidney. This process involves a series of ontogenetic events that are sequential and overlapping, resulting in the appearance and disappearance of what could be considered two transient kidneys. Three ontogenetic stages are commonly identified: the pronephros, mesonephros, and metanephros. These stages are of phylogenetic

interest because the pronephros bears a resemblance to structures that persist as the functional kidney in the amphioxus, the cyclostomes, and in larval forms of certain primitive fish. The mesonephros functions as the permanent kidney in most fish and amphibians (anamniotes). The final stage, the metanephros, persists as the final kidney in reptiles, birds, and mammals. The greatest importance of the early stages is their role as necessary precursors of the ultimate differentiation of the metanephros. Consideration of development should, accordingly, begin with the first step.

Development of the Kidney

Traditional embryologic teaching presents a taxonomic description of the early stages wherein major changes in gross appearance are used to separate renal organogenesis into three stages, pronephros, mesonephros, and metanephros. The prefixes *pro* (before), *meso* (in the middle), and *meta* (after or postalteration), are combined with the noun *nephros* to emphasize the chronologic order of renal ontogeny.

THE PRONEPHROS

The first evidence of a renal excretory system in the human embryo consists of individual paired tubules that arise from nephrotomes that form a hollowed-out vesicular structure called the *pronephric tubule*. The first tubules appear about the middle of the third week (8–9 somite embryo). They arise from a solid mass of mesenchymal cells located between the lateral surface of the somite and the coelom. The pronephric tubules persist for only a short time, and all have undergone regression by degeneration by the fifth week, which proceeds in a cephalocaudal sequence identical to the sequence in which the pronephric tubules appeared. A total of about seven pronephric tubules arise (somites 2 to 8). Each consists of a proliferating tubular bud that grows caudally until it fuses with the tubular bud of the pronephric tubule produced by the next nephrotome, giving rise to an important common structure, the pronephric duct, which persists after the pronephric tubules regress (Fig. 1-2*A*). These structures do not differentiate into recognizable nephrons even though collectively they constitute the so-called pronephros. The pronephros is of no significance as a functioning excretory organ since it has only a transitory existence. It is important because it gives rise to the mesonephric duct, which induces one of the unique tissues (the ureteric bud) that is required for the formation of the metanephros, the other being the nephrogenic blastema. The pronephros thus plays a primary role in normal organogenesis.

THE MESONEPHROS

The mesonephros appears immediately caudal to the last of the pronephric tubules (Fig. 1-2*A*). Vesicles now appear within the nephrogenic cords. The pronephric duct (now the mesonephric duct) continues growing toward the cloaca, adjacent and dorsolateral to each of the dense cell masses forming the nephrogenic cords. The mesonephros can be seen between the ninth and thirteenth somites in the 20-somite embryo. The first vesicles of the mesonephros differentiate at about the twenty-fourth to twenty-fifth day at the cranial end. Viewed topographically, the pronephros is a cervical organ, while the mesonephros is a thoracic organ. In all, about 40 pairs of nephrons develop between somites 9 and 29, with more than one adjacent to a single somite. As with the pronephros, the earliest nephrons formed (cephalic) begin to degenerate (fifth week), while the more caudal nephrons are still differentiating. The "organ" thus shifts caudally by this process. The majority of mesonephric nephrons have degenerated by the eleventh to twelfth week.

The mesonephric nephrons are the first true glomerulonephron units (Fig. 1-2*B*). Their formation is different from that of the pronephric tubules in that they arise by induction as differentiating vesicles within the solid cell mass of the nephrogenic cord that lies adjacent to the lateral collecting duct (the mesonephric, or wolffian, duct) which is already present. The individual vesicles differentiate into a primitive S-shaped tubule, and their distal projections establish continuity with the mesonephric duct (collecting duct). The proximal ends of these S-shaped tubules broaden and form a hemispheric two-layered cup. The outer layer forms the parietal layer of Bowman's capsule, and the inner layer forms the visceral epithelial portion of the primitive glomerulus. The glomerulus is vascularized by capillaries arising from branches of small arteries coming ventrolaterally from the aorta. The tubules differentiate into a definitive proximal tubule with convolutions and a brush border, but they have no loop of Henle or distal tubule. The glomeruli are larger than those in the metanephros, but the tubules are less complex. Collectively, they constitute the first true glomerular nephron collecting duct functional unit formed in renal ontogeny. The mesonephric nephrons remain actively functional during early development of the metanephros, so that for a time there is simultaneous function of nephrons in both structures.

There is great variation in different species in the functional contribution of the mesonephros during fetal development. In man and in the rat,

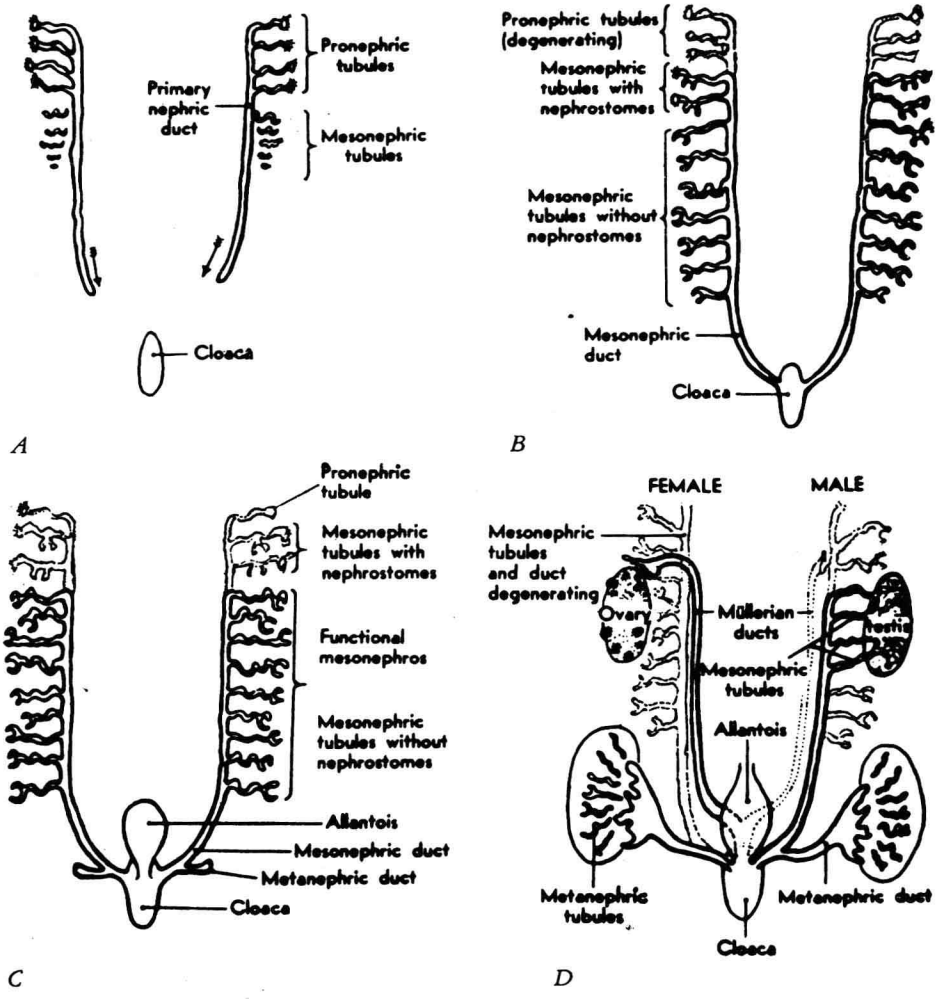


Figure 1-2. Relations of pronephros, mesonephros, and metanephros at various stages of development. To simplify, the tubules have been drawn as if they had been pulled out to the side of the ducts. The first rudimentary pronephros (A) is elaborated as a group of tubules emptying on either side into the primary nephric ducts, which extend caudad to discharge ultimately into the cloaca. A little later in development there arises a second group of tubules more caudal in position than the pronephric tubule. These are mesonephric tubules (A). In their growth they extend toward the primary nephric ducts and soon open into them (B). The plan, as shown in C, represents approximately the conditions attained by the human embryo toward the end of the fourth week. In D are depicted the conditions after sexual differentiation has taken place; female left side, male right side of the diagram. The müllerian ducts (shown in D) arise in human embryos during the eighth week, in close association with the mesonephric ducts. The müllerian ducts are the primordial tubes from which the oviducts, uterus, and vagina of the female are formed. Note that although both mesonephric and müllerian ducts appear in all young embryos, the müllerian ducts become vestigial in the male and the mesonephric ducts become vestigial in the female. (From B. M. Patten. Human Embryology *[3rd ed.]. Copyright © 1968 by McGraw-Hill Book Company. Used by permission.)*

it degenerates as the metanephros begins functioning, regressing completely by the eleventh to twelfth week; in the sheep, pig, and rabbit it becomes a prominent abdominal organ and contributes significantly to the formation of fetal urine for a variable time after the metanephros appears. Bremer's studies [6] of the interrelations between the mesonephros, allantois, and placenta in different species have demonstrated that the size and functional importance of the mesonephros is in general directly related to that of the allantois. The mesonephros and allantois are largest in forms with a simple appositional type of placenta and short lived in primates, in which the placenta is the highly permeable hemochorial variety and the allantois is rudimentary.

We have some idea of the functional ability of the mesonephros. Gersh [12] demonstrated elimination of ferrocyanide by glomeruli and phenol red by tubules in mesonephroi of the rabbit, cat, pig, and other animals from the time these structures first appear until they degenerate. Data on the composition of mesonephric urine in fetal pigs and sheep show that it consists of a glucose-free and protein-free fluid similar to an ultrafiltrate of fetal serum [24, 29]. It is isosmolar with body fluids, urea and solute concentrations being similar to fetal plasma. From studies of the mesonephros and metanephros in a number of anamniotes and avian reptile and mammalian forms, Davies [10] hypothesized that the metanephric kidney, with its capacity to dilute or concentrate urine, probably emerged in ontogenesis as the organism faced increased environmental needs to conserve water.

The mesonephros degenerates and ceases to exist as an excretory organ, but some of its tissues persist. In the male, the more cephalad tubules participate in the formation of efferent ductiles of the testis through which the seminiferous tubules communicate with the epididymis (see Fig. 1-2*D*). The latter arises from the mesonephric duct, as does the ductus deferens. In the female, some mesonephric tubules persist as minute functionless structures visible in the mesosalpinx at all ages and are known as epiopheron and paraoopheron (see Fig. 1-2*D*). These structures may become cystic and neoplastic.

THE METANEPHROS

The ultimate event in early renal ontogeny is the differentiation of the metanephros. It arises by the interaction of two different types of cells, epithelial and mesenchymal. Normal differentiation of the ureteric bud is essential for two basic organogenetic processes. The two processes are simultaneously operative but will be discussed separately. One is the initiation of branching, which leads to the formation of the urinary collecting system, beginning with the ureter and ending where collecting tubules are transformed into connecting tubules of nephrons. The second is the initiation of nephron formation, which occurs by chemically mediated interaction (induction) between the ampullae at the terminal ends of newly formed branches of the dividing ureteric bud and the contiguous metanephric blastema. This process will be described in detail later.

The ureteric bud arises during the fourth to fifth week of gestation as a small diverticulum at the caudal end of the mesonephric duct just proximal to the cloaca (Fig. 1-2*C*). Growth of the ureteric bud proceeds dorsally until it encounters the caudalmost portion of the nephrogenic cord. As the ureteric bud comes in contact with the nephrogenic blastema, it surrounds the growing and dividing ureteral bud as a condensed mass of cells (Fig. 1-3). These two tissues now in intimate proximity appear to migrate cranially. This change in position is coincident with rapid growth of the caudal somites and straightening out of the embryo from its curled configuration. The lower pole of the actual kidney remains at the same level as it arises, adjacent to the second or third lumbar vertebra. The developing kidney attains its final position after arising from the pelvis medially.

The consequences of faulty interaction in these early stages of renal organogenesis are serious. If the pronephric tubules fail to appear, renal agene-

Figure 1-3. Mesonephros, caudal extremity. U = ureteric bud; W = wolffian duct; B = nephrogenic blastema. Human embryo, 7 mm, thirty-seventh to thirty-eighth day. Institute of Anatomy Basle. (×105 before 28% reduction.) (From A. M. Du Bois. The Embryonic Kidney. In C. Rouiller and A. F. Muller [eds.], The Kidney: Morphology, Biochemistry, Physiology. *New York: Academic, 1969.)*

sis will result because there will be no structure (pronephric or mesonephric duct) from which the ureteric bud can develop. In the presence of such a basic defect in mesenchyme, however, the homolateral gonad is often absent, and the homolateral lung and adrenal gland may be absent as well. If there is renal agenesis and the lungs, adrenal glands, gonads, and sex ducts are all present and normally formed, renal agenesis would have to be attributed to failure of development of the ureteric bud or its faulty interaction with the metanephric blastema. If the ureteric bud and metanephric blastema do interact, malformations subsequently occurring will be associated with some glomerulonephron units, even if rudimentary. These distinctions may be useful when attempts are made to define the time of action of a teratogenic effect.

FORMATION OF THE PELVIS AND CALYCES AND INITIATION OF NEPHROGENESIS

The ureteric bud elongates and begins dividing about the sixth week, carrying with it the metanephric blastema as a cap. The first few bud divisions are not associated with induction of nephrons. The first formation of nephrons appears to be related to age rather than to the extent of branching. Evidence of nephrons developing simultaneously everywhere in the primitive kidney first appears at about 8 weeks. The nephron vesicles (first evidence of nephron differentiation) appear in direct proximity to the ampullae of the terminal branches of the dividing bud. The first nephrons observed by Potter [27] were attached to the third and fourth generation of the ureteric bud branches in the midpolar region and the fourth to sixth generation in the polar areas (Fig. 1-4). Shortly thereafter, ureteral bud branches proximal to the attachment of the earliest nephrons formed begin to dilate to form the primitive renal pelvis and major calyces (Fig. 1-5). Since this dilatation occurs after formation of the first glomeruli, it seems likely that it is related to the accumulation of urine.

Additional divisions of the terminal bud branches occur in rapid succession; they are associated with active nephron formation and allow little time for the new generations of tubules formed to elongate before dividing again. The pelvis and calyceal system expand and can be clearly demarcated from the profusion of branching tubules with clusters of attached nephrons (Fig. 1-6*A, B*). In the kidney of the 13- to 14-week fetus it is possible to make out the generations of ureteral bud branches that will form collecting systems, since they open into a calyceal chamber. The calyces are now evident as the expanded but nephron-free broad chambers subtending a confluence of later generations of nephron-bearing tubular branches (Fig. 1-7*A*). The primitive tubular branches emptying into each of the minor calyces will ultimately form papillary ducts. By 13 to 14 weeks the minor calyces and their communicating papillary ducts are well delineated and resemble those of the mature kidney (Fig. 1-7*B*).

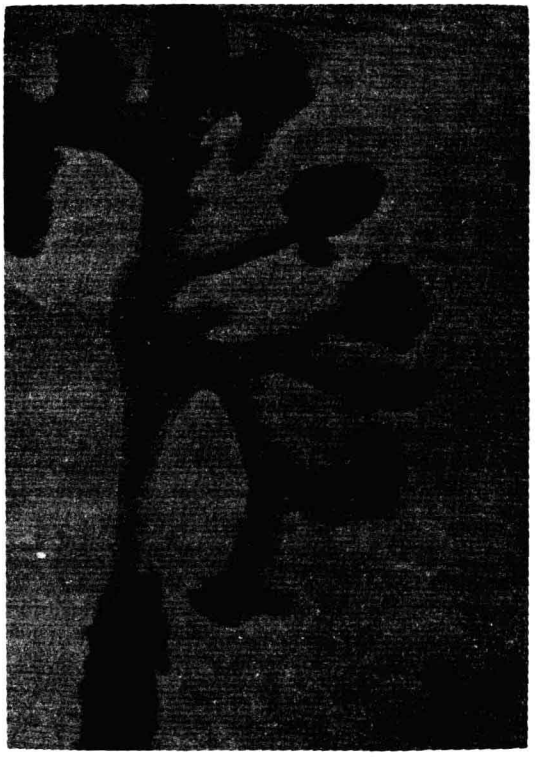


Figure 1-4. Microdissection of left kidney and ureter from 18-mm, 8-week embryo. Ureteral bud has divided four to six times in polar area and three to four times in interpolar area. All branches present at this stage will be expanded into renal pelvis and calyces during subsequent development. Renal vesicles derived from nephrogenic blastema are first present in relation to ampullary ends of third to fifth generation of ureteral bud branches. (From E. L. Potter. Normal and Abnormal Development of the Kidney. *Copyright © 1972 by Year Book Medical Publishers, Inc., Chicago. Used by permission.)*

FORMATION OF RENAL LOBES AND PAPILLAE

During early development the number of discernible lobes gradually increases. At about 10 to 11 weeks, these are only recognizable as a confluence

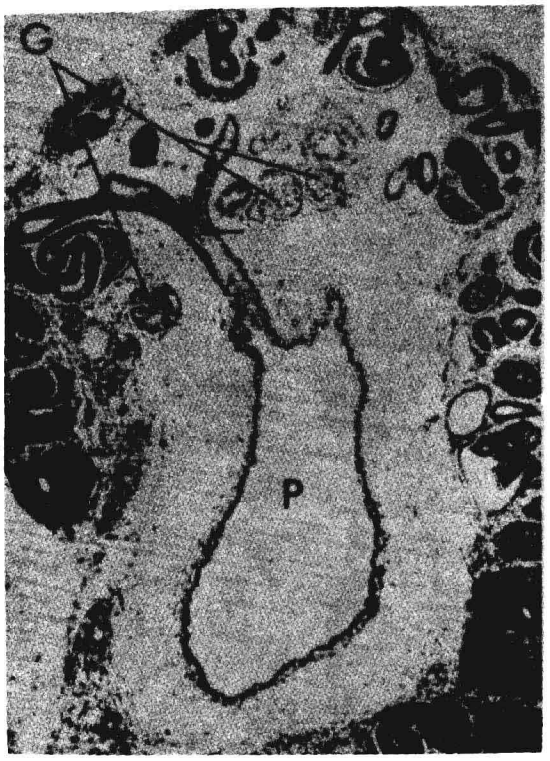


Figure 1-5. Kidney from 24-mm, 9-week embryo in cross section. Early branches of ureteral bud are dilated to form primitive renal pelvis (P). Dilatation of proximal portions of next generation will leave terminal portions constricted (indicated by arrow), through which calyces will communicate with pelvis. Of 18 well-formed glomeruli (G) present, 3 are visible in this section. (From E. L. Potter. Normal and Abnormal Development of the Kidney. *Copyright © 1972 by Year Book Medical Publishers, Inc., Chicago. Used by permission.)*

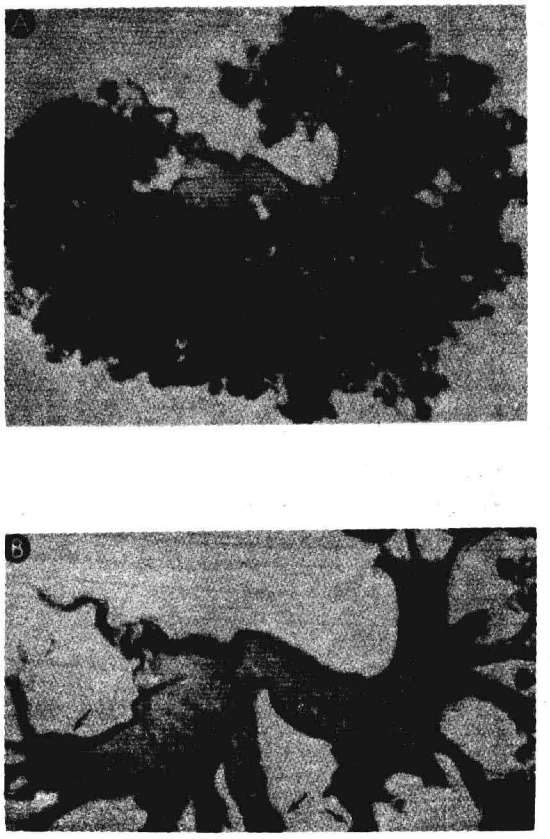


Figure 1-6. Kidney from 50-mm, 11-week human fetus. A. Entire kidney after preliminary microdissection. B. Most of the nephrons and collecting tubules have been removed to show ureter, renal pelvis, and earliest stages in formation of calyces. Tubules formed by third to fifth generation of ureteral bud branches have dilated to become primitive renal pelvis, and many large tubules empty directly into it. Initial evidence of calyceal formation is indicated by arrows. Note small diameter of ureter. (From E. L. Potter. Normal and Abnormal Development of the Kidney. *Copyright © 1972 by Year Book Medical Publishers, Inc., Chicago. Used by permission.)*

of a number of tubules communicating with a primitive calyx whose branches bear the first generations of nephrons that will persist (Fig. 1-6*A, B*). These primitive lobes consist of nephron-bearing, unexpanded tubular branches that are distal to, but connecting with, a nephron-free dilated system of tubular branches derived from the earliest generation of branches produced by the ureteral bud. The number of generations of ureteric bud branches that become incorporated into the formation of the calyceal system is variable, but this process sets the stage for the formation of lobes and determines the number that are formed. By 13 to 14 weeks the minor calyces have developed their distinct cup shape, and the viliform plate of the papilla is being formed (Figs. 1-6*B*, 1-7*A*). The latter ultimately consists of a series of tubular ducts (papillary ducts) entering into a common chamber (minor calyx). How the viliform plate is formed is unclear; Potter [27] ascribes it to a process of rapid growth of nephrons and ducts, resulting in compression that restricts expansion of generations of duct tubules produced distal to the last generation involved in calyceal formation.

Each renal papilla develops into a lobe, but the final number of papillae exceeds the number of calyces, since two or more papillae (compound) may enter one calyx (see Fig. 1-1*B*). The size of

A *B*

Figure 1-7. A. Kidney from a 74-mm, 13-week human fetus. Part of the ureter (lower right), interpolar portion of renal pelvis, and two minor calyces are shown, as well as the total length of a few collecting tubules and two nephrons. The calyces are more distinctively cup-shaped than in earlier stages and are beginning to assume a definitive form. Five to six generations of collecting tubules are present distal to those that dilate to form the renal pelvis. B. Kidney from 90-mm, 14-week fetus. The upper one-third of the renal pelvis and most of last few generations of collecting tubules and nephrons have been removed to show calyces and proximal portions of tubules (papillary ducts) communicating with calyces. (From E. L. Potter. Normal and Abnormal Development of the Kidney. *Copyright © 1972 by Year Book Medical Publishers, Inc., Chicago. Used by permission.)*

the lobe and number of nephrons formed within it vary directly in relation to the number of papillary ducts incorporated into the papillae. The orientation of each lobe is centrifugal with the terminal collecting duct branches extending peripherally to the primitive cortex. The renal papillae lie close together in a radial pattern around the pelvis. The areas within the kidney substance in which the outer surfaces of adjacent lobes are in apposition are designated the columns of Bertin. By the end of 16 weeks the papillae are sharply elevated and have an appearance similar to that of adult papillae. Developments responsible for the final shape of papillae and calyces take place during the fourth month. The number of lobes in the mature kidney (average 14 to 16) [27] are definitely established by the fourth month.

Development of the Urinary Tract

Differentiation of the urinary tract occurs synchronously with the early stages of metanephric development. At the fifth week, when the ureteric bud appears, the mesonephric duct communicates with the allantois and the cloaca (Fig. 1-8*A*). The urinary system separates from the primitive gut at about the sixth week, when the urorectal fold forms a septum isolating the urogenital sinus from the rectum (Fig. 1-8*B*). The formation of the bladder with a separate opening of the ureter from the mesonephric duct then occurs by a complex process that absorbs the terminal segment of the mesonephric duct into the bladder (Fig. 1-8*E, F*). The fate of the mesonephric ducts and tubules differ in the two sexes from here on. In the male (Fig. 1-8*E*), the most cephalad portion of the me-

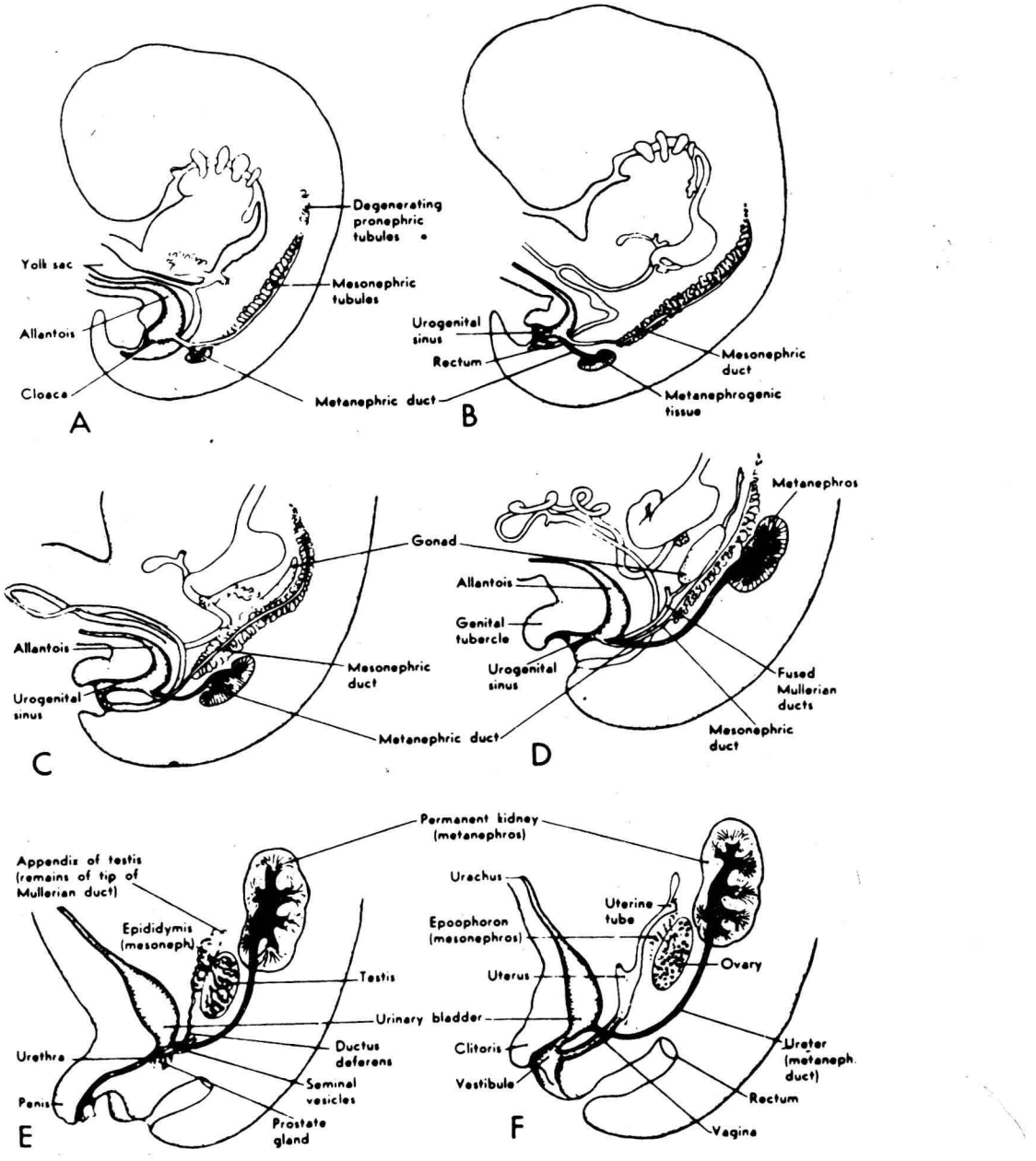


Figure 1-8. Relative sizes and positions of nephric organs of the human embryo at various stages of development. A. Early in fifth week. (Adapted from several sources covering 5- to 6-mm embryos.) B. Early in sixth week. (Modified from Shikinami's 8-mm embryo.) C. Seventh week. (Modified from Shikinami's 14.6-mm embryo.) D. Eighth week. (Adapted from Shikinami's 23-mm embryo and the Kelly and Burnam 25-mm stage.) E. Male at about 3 months, schematized. F. Female at about 3 months schematized. (From B. M. Patten. Human Embryology *[3rd ed:]. Copyright © 1968 by McGraw-Hill Book Company. Used by permission.)*

sonephric ducts (now the wolffian ducts) forms the highly convoluted duct of the epididymis, while the caudal portion forms the ductus deferens, ejaculatory ducts, and seminal vesicles. Some of the most cephalad mesonephric tubules persist as efferent ductules of the epididymis. The mesonephric duct degenerates in the female (Fig. 1-8*F*), but some of the mesonephric tubules persist as vestigial structures known as the ovarian rete.

The separation of the ureters from the wolffian ducts results in the formation of the trigonal area of the bladder, demarcated by the ureteral orifices and the veramontanum, where the ejaculatory ducts will enter. This occurs between the eighth and ninth weeks. The upper portion of the bladder is derived from the allantois. That portion above the bladder contracts to form a fibrous cord (urachus, Fig. 1-8*F*), except when lower urinary tract obstructions are present, when it may remain patent and allow discharge of urine to occur at the umbilicus (patent urachus). The fetal ureter does not open functionally into the bladder until about the ninth week (35-mm stage), which coincides with Gersh's [12] estimate of the time of onset of fetal renal function. Prior to this, a definite membrane is present at the anatomic junction of the ureter and urogenital sinus. Ureterovesicular obstruction and ureteroceles have been attributed to persistence of this membrane [8].

Bernstein [4] has summarized the considerable clinicopathologic and experimental data that provide evidence that fetal urinary tract obstruction can result in varied embryonic malformations involving all parts of the developing kidney—glomeruli, nephrons, the collecting system, and the pelvocalyceal system. The hydrostatic pressure generated by accumulation of urine formed by the metanephric kidney, prior to the normal opening of the ureter into the developing bladder, could play a role in the normal processes responsible for modeling the pelvocalyceal system.

Formation of the Collecting System

The collecting systems of the renal lobes in the mature kidney are highly systematized. The medullary portion is comprised of nephron-free branches that collect the urine formed by aggregates of overriding cortical nephrons. The cortical nephrons formed within a renal lobe are organized in multiple aggregates or clusters. All their nephronic tubules connect with individual branches of the collecting ducts that ultimately converge into a single papillary duct terminating on a single renal papilla. The centrifugal arborization of each of these collecting tree units (i.e. starting with a single papillary duct) reflects the process of collecting duct branching and nephron induction that occurred during the early stages of nephrogenesis described above. The connections of the nephronic tubules of the cortical nephrons to their collecting ducts are of two general types. The nephrons produced during early duct division have been transported to the periphery of the collecting-tree unit. These nephrons, which are deep-lying nephrons in the final cortex, connect to the collecting system by arcades of connecting tubules that, in a sense, overgrow the nephrons. There are also a larger number of nephrons whose attachments occur in series with the collecting-duct extensions of the terminal generation(s) of duct branches produced in the formation of each collecting tree. Oliver's [23] recent studies employing microdissection in fetal and mature kidneys have led him to the conclusion that each collecting tree is the result of a growth process that is regulated and determines the final number of collecting-duct branches in the system (Fig. 1-9).

The growth events responsible for elaborating the collecting systems found in the mature kidney can be understood best if the generations of tubular branches forming the papillary ducts in the cribiform plate in the mature kidney are considered as the first generation of ducts involved in formation of a collecting-tree unit. These tubules are the progeny of the fifth to seventh generation of branches derived from the primitive ureteric bud, counting the two branches resulting from the initial bud division as the first progeny. As previously mentioned, the first five to seven duct branches are incorporated into the formation of the pelvocalyceal system. Ducts distal to those incorporated into the calyces continue to divide, producing further progeny, until about 14 to 15 weeks; by this time seven to nine additional branches and generations of duct ampullae have been formed. The collecting-duct progeny produced by the seven to nine subsequent divisions of a single papillary duct constitute what Oliver defines as the *closed divided portion* of a single collecting system in the mature kidney.

After duct division ceases, growth of the terminal branches produces what Oliver calls the *open direct portion* of the collecting system. These terminal branches have all the nephron attachments in the mature kidney, either connecting directly to them or connecting via arcades, while the closed portion's branches are free of any nephron attachments.

The total number of generations of ureteral duct branches produced in forming the collecting-duct system is one of the major determinants of the final number of nephrons that will be formed, since all nephrons are induced by duct ampullae.

According to Oliver, the renal papilla of the average kidney has 44 pores, and the average number of papillae is 8. Accordingly, one can estimate that there would be 8×44 (352) individual collecting trees in the average kidney. Assuming (1) that the total number of branches each collecting tree comprises is the product of programmed division and each duct division is dichotomous, result-

Figure 1-9. Collecting tree at 5 months. The tubules have divided nine times in most instances, thus forming the fifteenth and final generation of the ductal system; i.e., its closed divided portion has thus been established. The divisions are now all bifid. All the nephrons have been transported (Peter's transport process) to the periphery, and a separation into cortex and medulla is apparent. The nephrons appear in all configurations, from vesicles to those with well-developed loops of Henle; the former occur only at the periphery on the terminal budding ampullae. Note that every nephron is attached to an ampulla either directly by its connecting tubule or by an arcade; none has lagged behind. The kidney contains about one-third the ultimate number of nephrons. The cortical region as it exists at this time will form only the juxtamedullary zone of the definitive cortex. (From J. Oliver. Nephrons and Kidneys: A Quantitative Study of Developmental and Evolutionary Mammalian Renal Architectonics. *New York: Hoeber Med. Div., Harper & Row, 1968.)*

ing in a doubling of duct branches, and (2) that each duct divides nine times, nine generations of duct branches would result in a total of 1024 branches in each collecting tree (geometric progression 1, 2, 4, 8, 16 . . . 512). Multiplying *total branches* by *number of collecting trees* (1024 × 352) gives an estimate of 360,448 total duct branches produced in the formation of the *closed divided portion* of the collecting system in the whole kidney. Assuming that each ampulla of each branch induced one nephron, and that all the nephrons produced moved to the periphery, this would account for about one-third of all the nephrons finally produced (approximately 1 million in each kidney), some 650,000 being produced later by induction on the nondividing branches of the last two generations of duct branches (fourteenth and fifteenth generations).

The quantitative results of the hypothetical program described provide a basis for a theoretical estimate based on accepted concepts of duct division and nephron induction. The actual number of divisions of the collecting-duct branches is more variable; Oliver [23] observed 709 in direct microdissection. He found reasonable agreement, however, between the total number of collecting-duct branches in the kidney derived from estimates based on actual counts by microdissection and the theoretical estimate. Potter [27] does not share his view of this process, and the subject remains controversial.

Branching of the collecting-duct system and

nephron formation continues actively until about 15 weeks, when it slows down, ceasing at about 20 weeks. The last generation of ducts formed (fourteenth to fifteenth generation from the ureteral bud) continues, however, to grow centrifugally after division ceases and continues to induce nephrons until about 32 weeks. Extensions of the terminal nondividing generation of collecting ducts grow toward the outer cortex in the form of spear-like tips. They form nephrons that will be found in the middle and outer cortex in the mature kidney. The nephrons located in the inner cortical region in the mature kidney (juxtamedullary) are those formed during the period of collecting-duct division. The surprising finding of microdissection in the mature kidney is that all the branches of the medullary portion of the collecting system, representing the seventh to thirteenth generation of ureteral bud branches, are nephron-free [23], (Fig. 1-9).

EXPLANATIONS FOR SEGREGATION OF NEPHRON ATTACHMENTS TO CORTICAL COLLECTING DUCTS

The connecting tubules of all the nephrons in the mature kidney are attached to the last two to three generations of collecting ducts produced by the divisions of the ureteral bud. As has been mentioned, the first functioning nephrons appear with attachments to the third to fifth generations of branches of the ureteral bud, and nephron induction continues on all subsequent branches formed in the early stages of development (8 to 15 weeks). There must, therefore, be an explanation for the ultimate segregation of the attachments of all nephrons in the mature kidney to the last two to three generations of the collecting tubules (thirteenth to fifteenth generation of ureteric bud branches).

There have been a number of explanations for the segregation of nephron attachments to cortical collecting ducts. These have included proposals that nephron attachments migrate or change during development, and that the earliest nephrons formed and left behind on the closed divided portion of the collecting system degenerate. The view most widely accepted holds that the segregation of all nephron attachments to the terminal collecting ducts arises as the consequence of a specialized growth process. It is understood that all nephrons are induced and form initial attachments only to ampullae. As the tip of an individual collecting duct divides to form new branches, an attached nephron could be carried forward with the advancing tip on one of the ampullae. This would have to occur with differential growth, allowing the attached nephron to move with one arm of the growing tip. In this manner it would also allow an accumulation of nephrons of different ages and at different stages of development to occur on one arm of a dividing and extending duct branch.

This process, originally proposed by Peter [25], is known as Peter's transport hypothesis and is shown diagrammatically in Fig. 1-10. It would (1) readily account for what appears to be movement of the attachment of nephrons formed prior

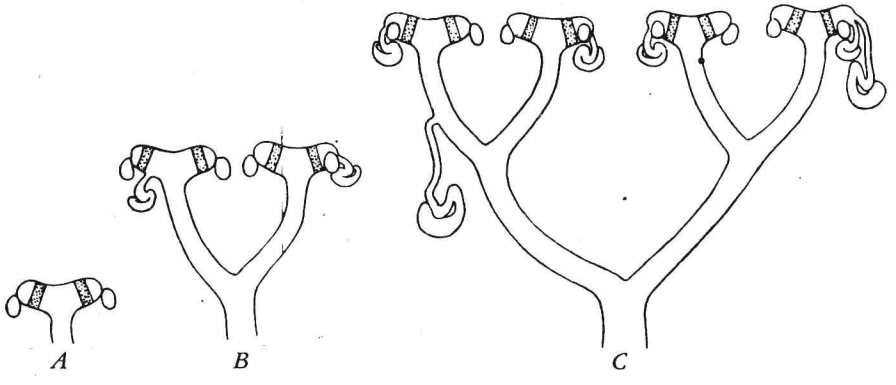


Figure 1-10. Peter's diagram showing his hypothetical intercalated zone of localized accelerated interstitial growth (dotted area) that carried the nephrons forward with the extending and dividing collecting tubules. To the right in A he has depicted the first generation of nephrons formed on the ampulla of a collecting tubule. After each generation of nephrons forms on the ampulla of a collecting tubule, it could be carried upward beyond the point of division if one assumes that an intercalated zone of accelerated interstitial tubular growth occurred below the attachment of nephrons (dotted area). To the left in B and C he shows one nephron that has been left behind. At the extreme right in C his concept of the origin of an arcade is shown. (From K. Peter. Untersuchungen über Bau und Entwicklung der Niere. *Jena: Fischer, 1909.)*

to new duct division peripherally to the new terminal branches; (2) explain the ultimate accumulation of the attachment of nephrons formed during the early stages of bud division on the terminal duct branches; and (3) result in the last generation of collecting ducts having varying series of attached nephrons in arcades.

Oliver [23] has utilized microdissection to demonstrate directly (1) movement of attachments of induced nephrons peripherally, (2) their consequent accumulation on later generations of bud branches, and (3) that the early branch on which nephrons first appeared later becomes nephron-free. He has published microdissection photographs of preparations showing nephrons attached to the seventh and eighth generations of collecting ducts in the 2⅛-month fetal kidney where these generations represented the terminal ureteral bud branches then present. On a later specimen (2½ months), where there were now 11 to 12 generations of bud branches, the seventh and eighth generations were nephron-free, and only the eleventh and twelfth generations (now terminal) had nephrons attached to them. The attached nephrons varied in stage of development, and the number on the different arms of the dividing terminal branches varied in complete accord with the predictions and expectations if (1) branching proceeded by dichotomous division of each bud, (2) each ampulla induced one nephron, and (3) Peter's transport process was operative during this period of formation of new branches of the collecting ducts. The nephrons produced during the period of duct division and "carried forward" account for less than one-third of total nephrons formed. They ultimately constitute the nephrons found in the mature kidney in the inner juxtamedullary cortex. Those nephrons whose attachments fail to move with the advancing ampullae eventually disappear. They can be found in various stages of degeneration close to the renal pelvis during fetal life and sometimes even after birth.

Nephrons in the middle and outer regions of the cortex in the mature kidney have different types of attachments to the collecting tubules of the collecting system than those of the deeper nephrons, which are connected by arcades. Urine secreted by the latter moves toward the periphery of the kidney via the connecting tubules of the nephrons before it enters a common collecting tubule and then flows toward the renal pelvis.

Potter [27] has proposed that the variable manner in which cortical nephrons connect to collecting tubules in the mature kidney is a result of three specific (programmed) changes in the growth activity of the ampulla and connecting tubules during renal development. During period 1, Peter's transport process is active. This lasts from the eighth to ninth week to the fourteenth to fifteenth week. She claims that formation of cortical arcades of nephrons continues after the transport process in period 1, but in a different manner because the terminal buds of the collecting-duct branches cease dividing as a result of a change in the growth program of the ampullae. Although they no longer actively divide, they maintain the capability of inducing new nephrons even though they have other nephrons attached. The connecting piece of the older nephron now shifts to communicate with the connecting piece of the younger nephron, and the older nephrons no longer move forward with the advancing ampullae. This is period 2 of nephron formation and extends from the fourteenth to fifteenth week to the twentieth to twenty-second week (Fig. 1-11). The result is a series of nephrons in a well developed arcade that drain into a single collecting tubule. This process seems sufficiently similar to that described by Peter to raise doubts about the justification for designating it as a separate process.

According to Potter, period 2 is in turn followed by another change in ampullary activity. During this stage, period 3, ampullae not only seldom branch but also now permit the direct attachment of nephrons to the growing collecting duct to persist *behind* the zone of active ampullary growth, thus no longer resulting in true arcade formation (Fig. 1-12). During this final period, nephron formation is usually repeated four to six times before ampullae cease inducing new nephrons [27]. As a result, the last nephrons induced are individually attached at regular intervals directly to the terminal portion of the last generation of collecting tubules formed during organogenesis.

The explanation proposed by Potter [27] of the mechanisms responsible for the variations in attachments of nephrons to the cortical collecting tubules of the mature kidney is still conjectural. An alternative explanation has been proposed by Oliver [23].

Oliver did find evidence indicating that a change in ampullary activity occurs at about twenty weeks that results in an entirely different orientation of subsequent nephron attachments, and he also relates this change to the cessation of branching. As a result, what he calls the open direct portion of the collecting system, located in the cortex of the mature kidney, is formed. The origin and subsequent modifications required to form nephrons during this second stage are similar to those in the preceding period, which involved formation of the closed divided portion of the collecting system and