# NEPHROGENESIS IN MAMMALS

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# INTRODUCTION

Nephrogenesis has been observed and described in detail only in a few mammals resulting in conflict in the interpretation of developmental anatomy and histology. A comprehensive, detailed study of nephrogenesis in a mammal other than a marsupial, rabbit or human is indicated for clarification of many points of conflict.

The animal selected was the dog, made possible by the accumulation of a series of serially sectioned canine embryos dated as days of gestation and somite number. Terminology from previous works has been selected to most clearly express and delineate morphology, primordial origin and potentiality, and yet conform as nearly as possible to current scientific usage.

# LITERATURE REVIEW

The development of the kidney in higher vertebrates has been a puzzle to embryologists since the first description. Histogenesis of most viscera — liver, heart, spleen, etc., evolves as a smooth, direct process from the initial anlage. In developmental processes certain organs appear, then de-differentiate without having become functional. Other organs fade into disuse in their original capacity and begin to degenerate only to have some part seized upon and salvaged by a new organ for some other function. Such is the case with the kidney (Patten, 1968); the permanent metanephros does not differentiate unless it is preceded by the successive formation of the pro-nephros, followed by the highly differentiated, and in certain species, func-tional mesonephros (DuBois, 1969). Both of these primitive urinary structures which seem at first sight to be mere abortive trials or sketches, in reality play an essential part in the inductive processes which determine the differ-entiation of the metanephros (DuBois, 1969).

During the evolution of Chordata, these excretory rudiments achieved completion and became functional — i.e., pronephros is the functional kidney of the very lowest vertebrates like Amphioxus, cyclostoma, and in the adult forms of elasmobranchs (Torrey, 1965). In its functioning, waste molecules from the capillaries of the glomerulus filter into the general coelomic fluid. Some of this fluid is drawn into the nephrostomes, whence it can be eliminated through the pronephric tubules and duct (Moog, 1949). The pronephros does not function in amniote embryos, consequently differentiation of the pronephric tubules in amniotes is quite variable. Since the tubules soon degenerate and disappear, the probable reason why they develop at all is that they are need-ed to initiate the development of the pronephric duct (Moog, 1949; Torrey,

1965; Patten, 1968; Balinski, 1970). The mesonephros which is the functional adult kidney of anamniote vertebrates is located caudal to the pronephros. It is also functional for a time in certain amniote embryos. Only the amniotes possess a metanephros, caudal to the mesonephric region of the body.

All three nephric organs are paired structures located retroperitoneally in the dorso-lateral body wall. In embryonic development of the mammalian urinary system, the pronephros, mesonephros, and metanephros appear in succession, furnishing an excellent epitome of evolutionary history (Patten, 1968).

The nephros of vertebrates consists essentially of an aggregation of tubular units known as nephrons (term coined by Braus, 1924), which are embryonically derived from the mesomere or intermediate mesoderm. Structurally this mesoderm becomes segmented with each such segment constituting the mesynchymal connection between the somite and the lateral mesoderm. The mesomere within the range of kidney development has been designated as the nephrotome (Torrey, 1965; Gier, 1970). According to Goodrich (1930), any given vertebrate nephrotome contains a coelomic chamber, the nephrocoele, which opens to the adjacent splanchnocoele by way of a so-called peritoneal funnel. The conversion of a nephrotome to a nephron involves the following major events: from the dorsolateral wall of the nephrotome arises a tubular outgrowth, the principle tubule, that communicates with the nephrocoele via a nephrostome (Goodrich, 1930); the medial wall of the nephrotome invests a tuft of arterial capillaries constituting the glomerulus, the wall itself being identified as the "renal capsule". In all vertebrates the nephrons empty into some variety of common drainage duct, i.e., the nephric duct (Fraser, 1950). This situation is complicated in modern higher vertebrates by reason of three variables. First, in the embryos of higher vertebrates, typical hollow nephrotomes are

seldom formed; instead nephrons differentiate without segmental arrangement within a continuous nephrogenic cord. Second, as the nephros develops embryonically, the entire kidney length does not appear at one time; rather, the nephrons appear in sequence from front to rear and the first-formed anterior tubules disappear before the posterior ones arise. Third, the nephrons become structurally more complex progressively, from anterior to posterior (Torrey, 1954).

Embryogenically, the tubules of all three excretory organs arise from intermediate mesoderm (Moog, 1949; Torrey, 1954; Patten, 1968), or as a thickening of the somatic layer of the intermediate cell mass or somite stalk uniting the somite with the lateral plate (Fraser, 1920; Balinski, 1970). Early in development the intermediate mesoderm loses contact with the somites. Later it loses its connection with the coelomic mesoderm except for sporadic strands (Patten, 1968). Caudal to somite eight or nine all trace of segmental arrangement is lost. It thus comes to constitute a column of cells which, because of its role in excretory tubule formation, is called the nephrogenic cord (Torrey, 1965; Patten, 1968).

Primary nephric duct formation becomes important in consideration of total kidney development (Torrey, 1965). Not only is it a drainage channel for the pronephros, if this organ functions, but even in the absence of definitive pronephrons, it is the first permanent unit of the nephric system to appear and subsequently becomes involved with the mesonephros on two scores: (1) it is the source of an inductive stimulus upon which the differentiation of the mesonephros largely depends; (2) then, having been joined secondarily by mesonephrons, it serves as the excretory duct for the organ which these nephrons comprise. Moreover, in amniotes, the primary nephric duct is the

source of the uteric diverticulum that initiates the metanephros and ultimately provides the ureter for that organ (Torrey, 1965). The primary nephric duct arises segmentally in the sense that it is formed by a caudal extension of each pronephric tubule until it meets and fuses with the tubule behind, thus forming a continuous channel (Patten, 1968; Balinski, 1970). The duct thus established continues to grow caudad by either (1) addition of new material progressively to it by differentiation in situ (Fraser, 1950; Burns, 1955; Fox, 1963); or (2) forcing of the tip of the cord posteriorly (migratory phenomenon), by terminal growth (Overton, 1959; Torrey, 1965; Gier and Marion, 1969).

The pronephros is the working kidney in the anamniote embryo and though it makes an attempt at development in mammalian embryos, it is an exceedingly transitory structure. In the canid embryo of 17-18 days, pronephric tubules form from nephrotome lateral to somite 7-10 (Gier and Marion, 1969). In human embryos, the pronephros is a rudimentary, nonfunctional kidney, which undergoes complete involution within less than two weeks (DuBois, 1969). Pronephric tubules begin to appear in embryos of 9-10 somites (Patten, 1968), or 8-9 somites (DuBois, 1969). In all, about seven pairs of poorly differentiated tubules develop from the nephrotome opposite somite 7-14 (Felix, 1912; Watt, 1915; Torrey, 1954; Patten, 1968; DuBois, 1969; Balinski, 1970). Only the three most anterior cervical primordia are completely separated from each other and show some metamerism (Felix, 1912). The most cephalic of these tubules, which are the first formed, are likely to show regressive changes, if they have not already disappeared, before the last in the series appear in 23-25 somite embryos (Patten, 1968; DuBois, 1969). From an anatomical point of view, the pronephros is a cervical kidney. Each nephrotome "hollows out", but the resulting vesicle fails to differentiate into a true nephron; its

external pole gives rise to a tubular bud, which grows out caudally and finally fuses with the bud of the next nephrotome. The tube thus formed, the pronephric duct, extends past somite 7 and finally reaches the cloaca by the end of the fourth week of gestation (DuBois, 1969). A detailed reinvestigation of very young human embryos by Torrey (1954), illustrated that classical nephrotomes are occasionally found in somites 1-6 (upper cervical region), in the form of small closed vesicles which fail to give rise to a tube, and rapidly degenerate. Beyond somite 7, clearly individualized nephrotomes are no longer found; instead there occurs a nephrogenic blastema, which from the start bears a longitudinal cleft dividing it into a narrow lateral portion, the nephric duct, and a larger medial portion, the nephrogenic cord, from which successively arise the seven nephrons which later open into the pre-existing nephric duct (DuBois, 1969). These rudimentary nephrons are comprised of a capsule with a flat visceral epithelium enclosing a vascular glomerulus. The periglomerular space (nephrocoeloma) opens at one end into the coelom through a narrow peritoneal funnel (nephrostome), and at the other end into an extremely short renal tubule leading to the nephric duct (Torrey, 1954). Beyond somite 7, and in later stages, the nephric duct progresses towards the cloaca. The nephrons of the mesonephros arise from the nephrogenic cord, which ultimately extends as far as somite 19. Thus in reality, the pronephros and mesonephros are but successive stages of a single morphogenic process (DuBois, 1969).

In the cat, rabbit, guinea pig, sheep, etc., the pronephros occurs as a small mass of nephrogenic tissue without recognizable nephrotomes (Torrey, 1954; Davies, 1950, 1951). The primordium of the excretory organ in the cat appears in embryos with 8-10 somites. The pronephric ridge arises as a thick-

ening of the somatic layer of the intermediate cell mass or somite stalk (Fraser, 1920; Balinski, 1970). This thickening begins in the region of the seventh somite and increases gradually in thickness to somite 13 or 14. During the same time as the formation of the ridge, in embryos with 9-17 somites, a series of coelomic chambers becomes cut off from the general body cavity (Fraser, 1920). They are well developed anteriorly, but from somite 9 posteriorly to the region of the shortened primitive streak, they form a progressive series most conspicuous in embryos of 12 somites. Fraser (1920), suggested that these are vestigal pronephric chambers. When best developed, each forms a well-defined chamber lying immediately ventrolaterally to the pronephric ridge and communicating with the general body cavity by a narrow passage, the peritoneal funnel (Fraser, 1920).

In older embryos the coelomic chambers disappear completely anterior to somite 10. From this level to the posterior end such coelomic chambers appear to become closed off from the coelom, and form a longitudinal cord of tissue, roughly circular in cross section, the entire cord being connected throughout its length with the coelomic epithelium by a short solid band of cells, representing the united and now closed peritoneal funnels. The cavities of the chambers become much reduced from somite 11 through 14, but apparently never quite disappear. Posterior to this somite level they become completely obliterated (Fraser, 1920).

The excretory system in the rat is initiated as a nephrogenic ridge, giving rise to the pronephros, from somite 9 through 12 in the 13-somite embryo but through somite 15 in slightly older embryos. In the 13-somite embryo, the intermediate mesoderm at the level of the posterior half of somite 9 appears somewhat condensed. At the tenth somite the condensation becomes more conspic-

uous, and its rounded dorsal surface is free from the overlying somite. Beginning abruptly at the level of the anterior end of somite 11, a second rounded cord-like structure appears on the dorsal surface of the intermediate mesoderm. This is the primary excretory duct which splits directly off the top of the ridge through the distance of the tenth through the twelfth somite and then progressively projects itself caudad by free terminal growth (Torrey, 1943). Before free terminal growth occurs in the older embryos, as the duct passes somite 12, it shifts slightly lateral toward its end, and then blends with the general nephric primordium. It contacts the cloaca in embryos of 24 somites and empties into the cloaca in embryos of 32-36 somites. Numerous funnel-like projections of the coelom protrude into the nephrogenic ridge through somites 10-12 and occur transitorily in 11-13 somite embryos. These are interpreted as vestigal pronephric tubules (Torrey, 1943).

Behind the pronephros the mesomere remains unsegmented and clusters around the pronephric duct. This tissue then produces, posteriorly from the pronephros, a series of mesonephric tubules which make openings into the pronephric duct or Wolffian duct (Moog, 1949). The mesonephric tubules differ from those of the pronephros in several respects: they are directly connected with the blood stream through internal glomeruli contained within the ends of the tubules themselves; nephrostomes either do not form or are transitory, so that there is no effective opening into the coelom; and several tubules occur per body segment (Moog, 1949). Also, the mesonephros is a much longer structure than the pronephros (Fraser, 1920; Moog, 1949; Torrey, 1954; Patten, 1968; Gier and Marion, 1969). Like the pronephric tubules, the mesonephric tubules are derived from the nephrogenic cord. The primary nephric duct has been indicated as the inducer of differentiation of the mesonephros (Torrey, 1965),

but there is a possibility that inductors other than the nephric duct may be variously involved, i.e. — chick and frog nervous tissue can induce mesonephromeres in competent mesonephrogenic tissue (Gruenwald, 1942; VanGeertruyden, 1946). In fact, Runner (1946), presented some evidence against the liklihood of induction of mammalian mesonephrons by the nephric duct. The mesonephric tubules attain a considerable degree of development and pending the development of the metanephrons are believed to be involved in active elimination of nitrogenous waste.

Gier and Marion (1969) indicated that the mesonephric duct in mammals is formed by fusion and posterior growth of the pronephric "tubules". The "pronephric duct", by lumenation, becomes mesonephric duct, posterior to the last pronephric tubule. In the 17-18 day dog embryo, *mesonephromeres* develop lateral to somites 11-27 and secondarily connect to the mesonephric duct, during days 18-23. Anteriorly, a single mesonephromere develops laterally to each somite, but posteriorly mesonephromeres develop independent of somites with up to four lateral to each somite, with a total of 52-55 mesonephromeres in the length of mesonephros spanning 16 somites. Each mesonephromere differentiates into one mesonephric corpuscle and one mesonephric tubule. The mesonephric ducts join the cloaca laterally near its anterior end, and remain ventral to the constriction of the gut and allantoic stalk, on the urogenital sinus. In the characteristic mammalian type of mesonephric tubule, no nephrostome is formed and the tubule receives all its fluid by way of glomerular filtration. There is considerable difference as to the degree of development attained by the mesonephros. For example, that of man, cat and guinea pig remains relatively small. The rabbit and pig, on the other hand, have very large mesonephroi. There is reason to suspect that the size attained by the mesonephros is in-

versely related to the excretory efficiency of the placenta (Patten, 1968).

In human embryos, mesonephric tubules begin forming during the 18-20 somite stage and later attach to the original pronephric duct (Patten, 1968; DuBois, 1969). The nephrogenic blastema is not segmented and extends from somites 9-10 (overlapping the caudal end of the pronephros) to somites 28-29 (DuBois, 1969). The young tubules, at first blind vesicles, soon make connection with the primary nephric duct after differentiating at the level of somite 14 (Torrey, 1954; Patten, 1968). Extension of tubule formation caudad from the point of its initiation is rapid and by five weeks it has reached its most caudad extent at the level of somite 26 (Patten, 1968). Two, three or more such vesicles develop alongside a single somite up to the ninth week of gestation reaching a total of approximately 80 (Patten, 1968; DuBois, 1969). Because the more cephalically located tubules are more advanced in their differentiation, a graded series of developmental stages may be seen by observing a parasagittal section, beginning caudally (Patten, 1968). During the fifth week the cranial nephrons start degenerating while the caudal nephrons are still differentiating. Thus from the sixth to the ninth week the mesonephros appears to be drifting in a caudal direction while at all times retaining its original length. This oblong structure bulging into the abdominal cavity is constantly comprised of 30-34 nephrons (Torrey, 1954; Patten, 1968; DuBois, 1969).

The development of the mesonephric nephron may illustrate in more detail the process of formation of individual tubules. When they are first budded off from the intermediate mesoderm, they appear as cell clusters close to, but not in contact with the nephric duct. These primordial cell groups become elongated and one end of each growing cell cord soon fuses with the duct. Once

they have attained connection with the duct, the originally solid tubules (DuBois, 1969), become hollowed and increase rapidly in length. Starting from a simple S-shaped configuration, their pattern is complicated by secondary bendings. DuBois (1969) indicated that the blind end of the S-shaped mesonephric tubule becomes invaginated to form the capsule of Bowman. This growth in length greatly increases the tubules' surface exposure, thereby enhancing their capacity for interchanging fluid and waste materials with the blood of adjacent capillaries (Patten, 1968; DuBois, 1969).

The mesonephros is fed by many small arteries arising ventrolaterally from the aorta. Each of these arterial twigs pushes into the dilated free end of a developing tubule, forming from it a double-walled cup called a glomerular (Bowman's) capsule (Patten, 1968). Within the capsule the artery subdivides into a knot of capillaries known as the glomerulus (Torrey, 1965; Patten, 1968; DuBois, 1969; Balinski, 1970). Blood from the glomerulus leaves the capsule through one or more vessels (efferent from the glomerulus), which again break up into capillaries and form a plexus in close relation to the body of the tubule in its tortuous course from glomerulus to duct. From these capillaries the blood passes to collecting veins which are for the most part peripherally located in the mesonephros and more or less circularly disposed about it. These collecting veins form a freely anastomosing system connecting with both the posterior cardinals and subcardinals, through which the blood is eventually returned to the general circulation (Patten, 1968).

The anterior end of the excretory organ in the cat obviously undergoes atrophy opposite somites 6 and 7. Neither in this region nor opposite somite 8 are any mesonephric tubules developed. Isolated tubular remnants are present opposite the ninth somite, and posterior to this level they gradually become larger and more definite. They are still rudimentary and few in number

opposite the tenth and beginning of the eleventh somite, but towards the hinder region of somite 11 they increase in size and the first internal glomeruli are apparent, although here the latter are always quite vestigial, so, the anterior end of the functional mesonephros is in the region of the twelfth somite (Fraser, 1920). In the cat there is no evidence of two kinds of tubules occurring in the same segment. The mode of origin of the mesonephric tubules follows that described in other forms. The dorsal wall of each vesicle becomes flattened and then invaginated to form the Malpighian Corpuscle, on the dorsal side of which the glomerulus arises. The solid connection between the vesicle and the Wolffian duct soon becomes tubular and coiled, and eventually develops into the secretory and excretory parts of the organ. The vesicles extending from the hinder level of the somites from 11-13, from which the duct takes its origin, give rise to tubules which are serially homologous with those behind this region; all are typical mesonephric tubules (Fraser, 1920). That being so, the distinction in the cat between pronephros and mesonephros regions is purely arbitrary, for no definite line can be drawn between the two areas.

The connection of the vesicles with the coelomic epithelium, representing the closed peritoneal funnels, persist for a considerable period. When the Malpighian body is developed, its ventral wall is connected with the coelomic epithelium either by a definite cord of cells, or by a diffuse mass of mesenchyme. In the cat, these atrophied funnels are still present in an embryo with about 55 somites, older stages not yet having been studied (Fraser, 1920).

In the rat, the next step of nephric development was reported (Torrey, 1943) from a group of embryos with 24-30 somites. Mesonephrons are alike in that all possess mesonephric tubules in various stages of differentiation.

Wolffian ducts, show varying degrees of canalization along its length and have reached the cloaca. Mesonephrons differ in that the numbers of tubules in process of formation range from 2 to 13 pairs. The mesonephros proper begins in all at somite 12, which is destined to mark the anterior limit for some time. Subsequent migration caudad results from relative differences in growth of parts of the embryonic body. The first mesonephric tubules appear at the level of the eleventh somite in the 24 somite embryo. The maximum number of 15-18 pairs of tubules occurs from 11 through 17 somites in 36 somite embryos. At this time, individual tubules are somewhat S-shaped and terminate in Bowman's capsules (Torrey, 1943). Histologically, the S-shaped tubule is differentiated only slightly along its length. At the distal end, its walls are composed of short columnar cells with elongate nuclei peripherally located. The cytoplasm of the basal portions of the cells is slightly more elongate, as are also their nuclei. The nuclei remain peripheral. There is little to indicate transition from the tubule to excretory duct.

The tubules in the 36 somite embryo are a little longer and the distal end of each is dilated as a Bowman's capsule. The peripheral wall of the capsule is composed of a uni-layered, cuboidal, lightly staining epithelium with nuclei centrally located. The adjoining tubule is composed of short columnar cells with peripherally located, darkly staining nuclei. The cells become even more columnar in the central portions of the tubule. There are no glomeruli associated with the capsules, a feature of the rat mesonephros that has been known (Torrey, 1943).

More significant is the relation of the mesonephric tubules to each other and to the Wolffian duct that prevails among the more anterior tubules. Instead of opening into the Wolffian duct independently, two or more may share

a common basal junction. Based on a wax reconstruction of the first 8 tubules, the first is a separate unit; the next two are joined at their basal ends and unite with the Wolffian duct through a common stem; the fourth, fifth, and sixth tubules likewise have common broad connection with the excretory duct. "The tubules decrease in number from posterior to anterior, lose their capsules, and combine into groups or complexes of 2-3 tubules each. These complexes subsequently become complicated by secondary branching." (Torrey, 1943).

When the metanephros is established, the mesonephros, regardless of the extent of development, undergoes rapid involution. The metanephros has a dual origin: part from tissue budded off from the mesonephric duct (ureteric bud), and part from the nephrogenic cord (nephrogenic blastema) caudal to the mesonephros (Torrey, 1943; Moog, 1949; Torrey, 1965; Patten, 1968; DuBois, 1969; Gier and Marion, 1969; Balinski, 1970). According to Torrey (1965), the uteric diverticulum emerges from the nephric duct near its junction with the cloaca and pushes forward into the rear of the nephrogenic cord. The distal end of this diverticulum enlarges as the primitive renal pelvis and coincidentally, the nephrogenic tissue, condenses around the pelvis as the metanephric blastema. Shortly afterwards, the pelvis exhibits a progressively greater number of subdiverticula, around each of which the blastema continues to condense. As the metanephric tubules differentiate from the metanephric blastema they open into the concomitantly elongating pelvic diverticula, which becomes the collecting tubules. The metanephrons and collecting tubules together comprise the definitive kidney, or metanephros, drained by the ureter derived from the proximal portion of the original diverticulum (Torrey, 1965).

The metanephric diverticulum originates during day 22 in the dog (Gier

and Marion, 1969). The mesonephric duct, formed dorso-lateral to the nephrotomes, passes ventro-medially around the nephrogenic cord posterior to the end of the mesonephros, and presses against and becomes fused with the nephrogenic cord lateral to the groove between somites 28 and 29. As the pelvic region is differentiating and expanding rapidly at this time, tension on the mesonephric duct results in a pocket, the metanephric diverticulum which is pulled from the dorso-medial surface of the mesonephric duct where it adhered to the nephrogenic cord. The effective tension on the mesonephric duct is provided by pelvic elongation, which results in both elongation and separation of mesonephric and metanephric ducts until by 28 days, they are separate to the urogenital sinus (Gier and Marion, 1969).

In human embryos as small as 5-6 mm the metanephric diverticulum can be identified as a tiny bud-like outgrowth just cephalad to the point where the mesonephric duct opens into the cloaca (Patten, 1968). Almost from its first appearance the blind end of the metanephric diverticulum is dilated, foreshadowing its subsequent enlargement to form the pelvis of the kidney. The portion of the diverticulum near the mesonephric duct remains slender, presaging its conversion into the ureter.

According to Patten (1968), the metanephric diverticulum arises at the level of somite 28, equivalent to the fourth lumbar vertebra. As it pushes out, it collects about its distal end mesoderm from the nephrogenic cord. The original relations of this mass of mesoderm are soon lost because it closely invests the pelvic end of the metanephric diverticulum and is pushed farther and farther away from its point of origin as the diverticulum continues to grow cephalad. Its change in relation should not lead to overlooking the fact that it was intermediate mesoderm like that which gave rise to the pronephric

and mesonephric tubules, only from a more caudal level in the body (Patten, 1968).

While the metanephric primordium is migrating cephalad, it increases rapidly in size and encroaches on the space previously occupied by the mesonephros. With rapid internal differentiation progressing, the pelvic end of the diverticulum expands within its investing mass of metanephrogenic mesoderm forming the major calyces as extensions both cephalad and caudad. These soon show subdivisions which are minor calyces. From the tip of each minor calyx there arise numerous outgrowths which push radially into the surrounding mass of mesoderm. These outgrowths become hollow, forming primary straight collecting tubules of the kidney. The group of straight collecting tubules associated with the minor calyx are the drainage channels for a natural unit of kidney structure. They, together with the tubules that develop from the immediately surrounding metanephrogenous mesoderm, constitute a renal lobe.

The changes in the metanephrogenic mesoderm which presage the formation of the uriniferous tubules occur near the growing ends of the terminal branches of the system of straight collecting tubules. The mesodermal cells become arranged in small vascular masses which lie adjacent to the blind end (ampulla) of the collecting tubule. Each of these vesicular cell masses is destined to become a uriniferous tubule draining into the straight collecting tubule adjacent to which it arises (Patten, 1968; DuBois, 1969). It has been amply demonstrated in experimental animals that this formation of metanephric tubules from the tissue of the nephrogenic cord depends on the inductive action of the metanephric diverticulum (Torrey, 1965; Patten, 1968; DuBois, 1969). The developing tubules extend toward the end of the collecting duct and soon the two become confluent (Patten, 1968).

According to DuBois' (1969) description, the uteric bud emerges from the dorsal aspect of the caudal extremity of the Wolffian duct, at the beginning of the fifth week on a level with somite 26. Its appearance is that of a small diverticulum, swollen at its extremity which at first extends dorsally and pushes into a mass of condensed tissue, the caudal most portion of the nephrogenic cord or nephrogenic blastema and then starts drawing it along in a cranial direction. This upward movement of the kidney from its original pelvic to its final lumbar position takes place between the end of the fifth to eighth week. The apparent spectacular ascent of the kidneys is essentially due to the straightening out of the embryo and to rapid longitudinal growth of its lumbar and sacral segments (DuBois, 1969). Upon emerging from the pelvis, as they rise above the common iliac arteries, both kidneys undergo a 90° rotation; the originally ventral hilus takes its final medial position, and the initially dorsal convexity becomes lateral. The first metanephric vesicles appear in the blastema at the end of the seventh week and promptly develop into nephrons (DuBois, 1969). Over 800,000 such nephrons are formed by week 32, at which time the neogenic activity of the blastema comes to an end (Osathanondh and Potter, 1963).

The material that follows will be merely to orient the origin of the permanent kidney of the rat in time and space as described by Torrey (1943). The mesonephric duct opens into the cloaca for the first time in embryos of 32-36 somites. Just before joining the cloaca the duct bends rather sharply towards the midline and enters the lateral side of the cloaca. This bend is at the level of somite 26, and it is at this bend where the ureteric (bud) primordium arises (Torrey, 1943).

The bud initially appears in embryos of 34 somites as a local thick-

walled dilation of the dorsal side of the excretory duct. From the first, the primordium is enveloped in the condensed tissue comprising the caudal end of the nephrogenic cord. This original primordium pushes out as a dorso-lateral bud which then turns cephalad, elongating all the while. In the 38 somite embryo the anlage has become transformed into a narrow tube with an expanded distal end which becomes the primary renal pelvis. The nephrogenous tissue surrounding the primordium becomes separated from the more anterior nephrogenic substance and remains as the blastema investing the primary pelvis (Torrey, 1943). In the 65 somite embryo (15 day), the anterior end of the metanephros will have pushed forward some three somites, i.e. — to somite 23, the final relative position assumed by the definitive kidney.

## MATERIALS AND METHODS

The embryos used in this study were obtained from two sources: laboratory dogs, and wild coyotes. Three sets of embryos were recovered from coyotes killed by hunters and received as part of the animals used in the study of "Factors Influencing Coyote Population" (Project 280, Kansas Agricultural Experiment Station). Most of the embryos were obtained from dogs maintained in the laboratory specifically for the study of reproduction problems and dog embryology (Project 321, Kansas Agricultural Experiment Station). These dogs were closely observed, ovulation time determined (Gier, 1960), and embryos removed by surgery at times calculated to provide the desired somite stages.

Fixation of the embryos was accomplished by one of three methods: (a) Bouin's fixative or formalin (routinely used), (b) in situ by perfusion of the fixative through the uterine artery of the excised tract, supplemented by injection of a small amount of 20% formalin directly into the embryonic vesicle, or, (c) the embryonic vesicle, supported by the endometrium, was stripped from the myometrium and fixed by immersion. After fixation the embryos that had been fixed in the uterus were dissected out, and in some cases, if they were small enough, were sectioned with a segment of the uterus. Part of the embryos were stained with either dilute acetocarmine or with Harris' haematoxylin diluted 1 to 20. These embryos were then dehydrated, embedded in paraffin and most of them sectioned at 10 or 12 microns. Selections were made to allow at least one whole mount, one transverse series, one longitudinal series, and one diagonal series in each critical group. The sections were stained in Harris' haematoxylin (125 ml Harris' haematoxylin and 8 ml acetic acid diluted to 500 ml with water), and counterstained with either eosin or a mixture of orange G and acid fuchsin.

The youngest embryos used in this study were removed late in the 16th or

early in the 17th day of gestation. The oldest embryos studied in detail were removed from the uterus during the 28th day of gestation, at which time the metanephros was sufficiently advanced anatomically to suggest functional activity.

The age of the embryos was determined within a two day period by calculation of gestation time from ovulation (Gier, 1960). Because there is a variation of approximately 8 somites in one day of development and in some cases ovulation time had not been accurately determined, other criteria were used for staging of embryos not critically timed. Carefully staged embryos, according to a derived standard curve, had 2 to 6 somites at the end of the 16th day after ovulation, 25-35 during the 20th day, and reached the full compliment of 54 somites on the 26th day. Somites were readily counted both on whole mount preparations and on serial sections up to the 30 somite stage, after which the anterior somites became differentiated enough that they were difficult to identify. By this time the spinal ganglia clearly marked the intersomitic grooves and the posterior edge of the hind limb bud covered the 30th intersomitic groove, so it was necessary to count only the somites posterior to the limb bud.

For convenience of study, the embryos were divided into three groups (Table 1). Group I contained 6 embryos ranging from 7 to 26 somites and showed nephrotome and pronephric differentiation. Group II, with 16 embryos, showed the establishment of the mesonephros and progression of the nephric duct to the cloaca and its connection with it. Group III contained 11 embryos and showed the initiation of the metanephric diverticulum, and the establishment of the primitive ureter, the primitive pelvis, and the metanephric blastema with its Bowman's capsules and major and minor calyces.

Table 1. EMBRYOS STUDIED

| Group | Designation | Number of Somites | Age (Days) | Sections Tran. | Sections Long. | Thickness in Micra |
|---|---|---|---|---|---|---|
| I | 121R | 7 | 17 | | X | 10 |
| | C1841 | 8 | 17 | Whole Mount | | —— |
| | 77L | 17 | 17+ | X | | 10 |
| | 116Rb | 19 | 17+ | | X | 10 |
| | 121L | 20 | 18 | X | | 10 |
| | 45L | 25–26 | 18+ | | X | 18 |
| II | 116Rb | 19 | 17+ | | X | 10 |
| | 121L | 20 | 18 | X | | 10 |
| | 45L | 25–26 | 18+ | | X | 18 |
| | 42L | 28 | 19 | X | | |
| | D354R | 36 | 21 | X | | 12 |
| | 49L | 34–35 | 20–21 | X | | 10 |
| | 81Ra | 35–36 | 21 | X | | 15 |
| | 254 | 35–36 | 21 | | X | 10 |
| | 81R | 35–36 | 21 | X | | 15 |
| | D358LA | 35–36 | 21 | | X | 8 |
| | 48R | 36 | 21 | X | | 15 |
| | 241LA | 47 | 24 | X | | 15 |
| | 83LC | 47 | 24 | X | | 10 |
| | 145LC | 48 | 24 | X | | 15 |
| | C953 | 50 | 25 | X | | |
| | 130RD | 54 | 26 | X | | 15 |
| III | D354R | 32 | 20 | X | | 12 |
| | 49L | 34–35 | 20–21 | X | | 12 |
| | 48R | 36 | 21 | X | | 15 |
| | 123RC | 45 | 23 | X | | 10 |
| | 241LA | 47 | 24 | X | | 15 |
| | 145LC | 48 | 24 | X | | 15 |
| | C953 | 50 | 25 | X | | 10 |
| | 46L | 54 | 26 | X | | 25 |
| | 60R | 54 | 27 | X | | 15 |
| | 130RD | 54 | 26 | X | | 15 |
| | D9L | 54 | 30 | | X | 25 |

# OBSERVATIONS

## Group I

## Embryos from 7-26 Somites

The youngest embryo used for critical study was a 7 somite dog (121R) serially sectioned longitudinally at 10 micra. In this embryo, intermediate mesoderm showed no differentiation from the first through the sixth somite. At the medial one-third of somite 7, a dorso-lateral swelling from the nephrotome was observable for three sections.

In the 8 somite whole mount coyote embryo (C1841) nephrogenous tissue was detectable lateral to somites 4 to 7. Again, a swelling interpreted as pronephric "tubule" was observed on the nephrotome lateral to somite 7 (Fig. 1).

Lateral to somites 2-5 inclusive, in a 17 somite embryo (77L) sectioned transversely at 10 micra, the mesomere was broken into mesenchyme. At the anterior end of somite 6, a nephrotome was observed (Fig. 2). Lateral to the anterior portion of somite 7, the first pronephric "tubule" was observed accompanied by a splanchnocoelic depression (Fig. 3) immediately lateral to the nephrotome. As the pronephric "tubules" formed, a cord outgrowth from the nephrotome rather than a "pinching off" was observed. The nephrotome as such had disappeared at somite 7 and anteriorly where the derma-myotome was distinctly differentiated. At somite 8 the pronephric cord was observable over the pronephric "tubule" (Fig. 4), while rudimentary nephrostomes were present lateral to the centers of somites 8 through 11, connected to the coelomic epithelium (Fig. 5). At somite 9 a nephrostome and pronephric "tubule" were observed together (Fig. 6). The posterior tip of the pronephric cord and the last pronephric "tubule" were lateral to somite 11 (Fig. 7), where the pronephric cord reached its maximum. At somite 15 the nephric duct terminated

over the undifferentiated nephrotome, and further caudad, nephric structure was limited to nephrotomic material only.

Pronephric remnants were present at somite 11 in the 19 somite embryo (116RB). Mesonephromeres were developing lateral to somite 12, only.

The pronephric region was extended from somite 7 through 11 with vestigal pronephric tubules at somites 9 and 10 and intact "tubules" at somite 11 in the 20-26 somite embryos (121L, 45L). Pronephric "tubule" was present at somite 7 as a solid cord. Nephrostomes were visible lateral to somites 8 through 10 in a 20 somite embryo (121L) and between somites 10 and 11, and 11 and 12. The end of the pronephric "tubules" was at the 11th intersomitic groove and the beginning of the mesonephromeres at somite 12 (Fig. 9), with the mesonephric duct dorsal to them.

## Group II

### Embryos from 19-54 Somites

The first mesonephromeres were found just behind somite 11 and were developed only along somite 12 in a longitudinally sectioned 19 somite embryo (116RB). The nephric duct extended posteriorly over the nephrotome lateral to somite 14.

Mesonephromeres were distinct at the 11th intersomitic groove, posteriorly to the 17th somite in the 20 somite embryo (121L). At somite 13 a mesonephromere was secondarily attached to the overlying nephric duct, illustrating root growth rather than delamination. The pronephric duct grew posteriorly independent of the nephrotome. The vitelline artery occurred as 6 or 7 pairs of trunks attached to the dorsal aorta from somites 16-24.

The nephric duct extended more caudad, reaching the cloacal region in

longitudinal sections of a 25-26 somite embryo (45L), as a solid structure all the way with no indication of a lumen (Figs. 10, 12). A lateral outgrowth from the wall of the cloaca protruded towards the nephric duct but had not connected with it. The mesonephromeres were present from somite 12 caudally to the segmental plate; the anterior ones along somites 12 to 15 were cavitated and were connected to the nephric duct by solid cords. There were three or four mesonephromeres per somite beginning at somite 12, showing no set relationship of mesonephromeres to somites.

In this 36 somite embryo (D354R), mesonephromeres were differentiated to somite 22 and the mesonephric duct extended back to the cloaca (Fig. 28) with complete lumenation of the duct. Segmental arteries supplied individual mesonephromeres (Figs. 19-21) with each artery supplying 3, 4, or 5 glomeruli (Figs. 23, 24). Glomeruli were always found medially, near the dorsal aorta, in the mesonephroi.

The pronephros had disappeared in the 35 somite embryo (49L). The anterior tip of the nephric duct, over the first mesonephromere, was dorsal to the lung bud. Progressive stages of glomerular formation were present, with long-tapered vasofactive cells in the medial curve of the sigmoid mesonephromere (Fig. 16), forming the vessels of the glomerulus. Four mesonephromeres per somite were present from somite 22 through somite 26. All mesonephromeres were connected to the nephric duct which was fully lumenated throughout its length. Mesonephromeres showed successive stages of development from posterior to anterior, with newly formed mesonephromeres at the level of somite 26, cavitated mesonephromeres at somite 18, and progressive stages of sigmoid development from somites 17 to 12. The sigmoid flexion occurred in that portion of the elongating mesonephromere which connected the mesonephric corpuscle to

the nephric duct. This connecting portion was lumenated only at the proximal part nearest the corpuscle. The capsule with the developing glomerulus was directed dorsally with a slight turn medially.

Cranially, the nephric duct in the 35 somite embryo (49L) was dorso-lateral to the mesonephros, but caudalwards, the duct assumed a lower position and became ventro-lateral to the posterior cardinal vein and lateral to the mesonephromeres (Fig. 17). The duct connected to the cloaca in the same area where the allantois joins the hind gut. This duct connects with a definite part of the hind gut, the urogenital sinus, which extended the length of somites 30 and 31.

Dog embryo series of 35-36 somite stage (81Ra, 254, 81R, and D358LA), showed mesonephric tubules traversing the lumen of the posterior cardinal vein from the corpuscle to the mesonephric duct, with three tubules per somite length, beginning a separation of the ventral, subcardinal vein from the dorsal, posterior cardinal vein. There was one mesonephric artery per somite, in a position corresponding to the intersomitic groove and the middle of each ganglion. The cavitated mesonephromeres showed exceptionally clearly (Figs. 11, 13) in one longitudinally sectioned embryo (D254) with 3 to 4 per somite which have begun to expand laterally over the mesonephromeres. Mesonephromeric cavitation was maximal at somite 16, although present to somite 23. All the mesonephromeres formed were connected to the mesonephric duct. No remnants of pronephros were visible in this or other 36 somite embryos. The anteriormost tip of mesonephros was at somite 14-15. The tip of the nephric duct was 40 micra (four cell lengths) anterior to the first mesonephric tubule, at the anterior edge of the liver. The posterior cardinal vein extended down between the mesonephric tubules, which appeared to extend out into the lumen

of the vein, forming "mesonephric arches". Every 40-50 micra along the length of the posterior cardinal vein from somite 14 through somite 23 inclusively, these mesonephric arches were well developed, with a Bowman's capsule at the medial end of each mesonephric tubule surrounding a glomerulus which was connected to a segmental artery. Longitudinal sections of one of the 35-36 somite embryos (D358LA) showed conclusively that the mesonephros extends from somite 12 to 26 with glomeruli and corpuscles well developed, on all tubules. The anterior end of the mesonephros was beginning to expand. The mesonephric duct was dorso-lateral to the mesonephros as far back as somite 25 (Fig. 42).

Segmental arteries from the dorsal aorta each supply glomerular tufts of 3 or 4 mesonephric corpuscles (Figs. 22, 23). One 36 somite embryo (48R), sagittally sectioned, demonstrated particularly well the multiple branching of the segmental arteries although such was seen in all embryos of this stage.

In embryos of 47 somites (241LA and 83LC), no mesonephric structures were found along somites 12 or 13 although there was no indication of necrosis in the mesonephromeres. Mesonephric duct and tubules had receded to somite 15. Mesonephric tubules traversed the posterior cardinal vein, as was seen in other embryos of 35-40 somites. Mesonephric corpuscles with glomeruli were found only anterior to the anterior-most vitelline artery at somite 16 or 17 (Fig. 14). Mesonephric veins from the glomeruli to the posterior cardinal vein were distinct, as were subcardinals ventro-lateral to the dorsal aorta. Glomeruli were in different stages of development, more progressed than the vasofactive cell stage. The genital ridge has become pendant below somites 18 to 24 (Figs. 23, 25).

By the 48 somite stage (145LC), the last mesonephromere has formed ventro-

medial to the mesonephric duct ventral to somite 26 at the anterior limit of the hind limb bud area (Fig. 28).

By the time the somite numbers were complete (54 somites, 130RD) at 26 days, the first mesonephric tubule joined the mesonephric duct posterior to the lung bud, ventral to somite 15, indicating a posterior movement of the anterior tip of the mesonephros by a full three somites (Fig. 15). The first glomerulus was just posterior to the lung bud. There were 38 or 39 developed and apparently functional glomeruli in the mesonephros at this stage.

## Group III

## Embryos of 30-54 Somites

The last mesonephromere was found at somite 26 in the 30 somite embryo (D354R) and older stages, while the metanephrogenic tissue (nephrogenic cord) extended from somite 26 to 30. The hind limb bud extended the length of four somites, with its back edge at somite 30. No metanephric bud was found in the 30 somite embryo; rather the mesonephric duct was wrapped around the nephrogenic cord between it and the leg bud ventro-laterally, making a point contact, then running parallel to the cord under the iliac artery (Fig. 29). The contact point between mesonephric duct and nephrogenic cord is the metanephric anlage in which the metanephric blastema develops in the nephrogenic cord and the metanephric pelvis from the mesonephric duct. The metanephric anlage was 0.25 mm or almost two somite lengths posterior to the last mesonephromere.

By the 36 somite stage (48R), a metanephric blastema was differentiated from the nephrogenic cord and a metanephric pelvis pulled out from the mesonephric duct (Figs. 32, 34).

In the 45 somite embryo (123RC) the metanephros was lateral and posterior to the iliac artery. Posterior to the metanephros was a mass of nephrogenic cord which later becomes gubernaculum (Fig. 33).

In embryos of 47 and 48 somites (241LA, 145LC) the renal pelvis had formed as an expansion of the metanephric duct within the blastema, and the metanephric duct had elongated as a rather narrow tube (Fig. 40). The developing metanephros was immediately posterior to the last mesonephromere (Fig. 37). Both metanephroi had attained equal stages of development on the left and right side of the 48 somite embryo (Fig. 38).

By the 54 somite stage (46L, 60R, 130RD, and 9L), the metanephric pelvis was undergoing branching into calyces and papillae (Fig. 39). The metanephros and metanephric duct were ventro-lateral to the double dorsal aortae and were differentiating and elongating rapidly as indicated by stages only slightly advanced in other structures (Fig. 35). Posteriorly, the entrance of the mesonephric duct into the urogenital sinus completed the connection between the permanent kidney (metanephros) and the excretory orifice (Figs. 26, 27). At its largest size in this stage (9L), the metanephros was 285 micra in length and the metanephric duct was 150 micra to the point where it joined the mesonephric duct. There was a 185 micra length of common duct between the junction of the mesonephric and metanephric ducts and the entrance of the mesonephric duct into the urogenital sinus. At the same stage the metanephric blastema was differentiating, and well developed Bowman's capsules and calyces were establishing the basic structures to complete the formation of the permanent kidney (Fig. 41).

# DISCUSSION

The cranial end of the excretory system in the dog begins as a real nephrotome at somite 6 as the mesenchymal connection between the somite and the lateral mesoderm. This differentiates into a pronephric ridge, as a dorsal thickening of the nephrotome lateral to the somites.

Fraser (1920) and Balinski (1970) both stated that the nephrotome from which the pronephros arises is a stalk of the somite. In the dog there was found no indication of a "somite stalk".

## The Pronephros

The pronephros, which differentiates into an observable structure in embryos with 8-9 somites, starts in the region of the seventh somite and increases gradually in thickness caudalwards to somite 10 or 11 in older embryos. Considering the pronephros to be the cervical kidney created two problems, one of which was formation of the pronephric units. The other was the formation of the pronephric duct, the mechanism of its initiation, and the factors involved.

In the work done by many investigators on the nephric development in different animals, two interpretations of the origins were proposed. The first method which several works (Moog, 1949; Torrey, 1965; Patten, 1968; Gier and Marion, 1969; Balinski, 1970) favored is summarized as follows: (a) definite pronephrons appear from the dorsal surface of the nephrotome, (b) the pronephrons grow dorsally and then turn caudad, (c) their distal parts fuse, forming, (d) a continuous structure (tube or cord), the pronephric duct, (e) which continued to grow caudalwards by independent terminal growth until it reached and connected

with the cloaca.

This method requires the development of definite pronephrons and their participation in the formation of the pronephric duct. Pronephrons in several animals (guinea pig and rabbit, rat, and human) had tubular structures and were spoken of as pronephric tubules by their authors (Weinburg, 1929; Torrey, 1943; Patten, 1968; DuBois, 1969). Such was not observed in the dog as the pronephric "tubules" were solid cords of cells throughout their entirety. When the longitudinal pronephros of certain animals has cavities, or nephrocoels, connected to the splanchnocoel by peritoneal funnels, or nephrostomes, it was designated as the pronephric duct, or segmental duct because of participation of several elements in its formation.

Torrey (1943), stated that among those amniotes in which a pronephros is known to occur, it is a relatively rudimentary structure. Patten (1968) and DuBois (1969) also stated that the pronephros in the human is very transitory and that as a multinephron it is found only in the very lowest mammals. Curiously enough a multinephron pronephros similar to that of the human embryo is found only in the Echidna and Trichosaurus (Australian opossum). In cats, rabbits, guinea pigs, sheep, etc., the pronephros remains a small, undifferentiated mass of nephrogenic tissue without recognizable "nephrotomes" (Torrey, 1954; Davies, 1950, 1951). The pronephros of the cat was described by Fraser (1920) as consisting of little more than a pronephric ridge, extending over some seven somites, associated with a series of vaguely defined, isolated coelomic chambers. These chambers were homologized with pronephric tubules, although her principle reason for calling this region the pronephros was that it is the site of origin of the nephric duct. These authors gave the impression that the pronephros is very unimportant. In the dog, pronephric "tubules" are

definite and play a positive role in the formation of the pronephric duct.

A good example of the first method is represented by the Gymnophionan, Hypogeophis, described by Brauer (1902). In the embryos of this animal there were 12 tubules. The first three tubules participated actively in the formation of the excretory duct, and the rest, which complete their growth (5-8, inclusive), connected to the duct secondarily. Similar descriptions for the human were given by Felix (1912), Johnson (1917), and Watt (1915). The same method of pronephric formation was described by Hamburger and Hamilton (1951) in the chick where the pronephric tubules, formed from somites 11 to 15 inclusive, participated in the initiation of the pronephric duct.

The second method considered was that of splitting and delamination as described in the lower vertebrates such as anura (Camber, 1948) in which no definite pronephrons are formed. The nephrotomic plate, in the pronephric area, showed a continuous dorsal ridge-like growth, called the "nephric ridge". After the ridge was established, there followed a distal splitting by which the dorsal part was delaminated from the mass proper. Beyond this area the posterior tip of the delaminated structure continued to grow caudalwards independently.

Fraser (1920) reported that the nephric ridge of the cat extended from somite 9 through 14 inclusive. The pronephric duct was described as developing secondarily by delamination of the dorsal part of the ridge and then continuing caudalwards by independent growth. A comparable method was described in human embryos by Wen (1928), Atwell (1930), and Heuser (1930), who reported that the pronephric duct originated by differentiation in situ. Torrey (1954) confirmed this method in human embryos with the observation that the pronephric duct originated by in situ differentiation and delamination from the nephrogenic cord

in the range of somite 9 to 13 or 14 inclusive. Posterior to this range the developing pronephric duct continued caudad by independent terminal growth.

In the dog, the first pronephric "tubule" originates from the nephrotome at somite 7, extends dorsally toward but not to the ectoderm, and then turns caudad to the range of the eighth somite where it connects with the succeeding "tubule". These "tubules" are connected to the splanchnocoele by a solid peritoneal funnel called the "nephrostome". The second pronephric "tubule" originates from the nephrotome at the level of somite 8 and grows dorso-caudad fusing with and overlapping the preceding "tubule" at the middle of the somite. This structure then extends caudalwards as a cord of cells where it is connected to succeeding "tubules" of somites 10 and 11. Thus a common structure, the "pronephric duct", originated indicating that the first method was expressed in canine nephrogenesis by the participation of the last three "tubules". After it was formed from fusion of "pronephric tubules", the pronephric duct continued caudalwards over the underlying nephrotomic tissue (Fig. 8). Several authors (Meyer, 1890; Felix, 1904; Hertwig, 1910) stated that the pronephric duct was formed by delamination from the ectoderm, but nothing comparable was found in the dog.

The classical notion of the cranial part of the pronephric duct arising through budding and successive fusing of pre-existing nephrotomes was not adequate. As pointed out for the dog, formation of the pronephric duct by outgrowths from the nephrotome seems the better one.

The nephric duct in the dog reached the cloacal region when the embryo was in the 25-26 somite stage, coming into approximation with an evagination from the lateral side of the cloaca. In the 28-29 somite stage, the nephric duct had established its connection with the cloaca evagination. The nephric

duct up to that stage was a solid cord of cells extending from the pronephric area above the nephrogenic tissue. As it approached the hind gut, the duct made a downward curvature to the urogenital sinus in the segmental plate area. It began to show a lumen at the 21 somite stage and became fully lumenated at the 35 somite stage.

The nephric structures differentiate and elongate regardless of what the somites are doing. The somites are used strictly as a reference point in nephrogenic development. In the canid preparations studied, no indication of splitting of the somite to form a myocoele was found except a few artifacts from dehydration and violent shrinkage during histological preparation. No real connection was found to exist between nephrogenesis and somite differentiation except the space relationships.

A reinvestigation by Torrey (1954) of very young human embryos brought to light several points one of which has just been refuted by this author. Torrey stated that true classical nephrotomes are occasionally found in somites I-VI (upper cervical region) in the form of small closed vesicles which fail to give rise to a tube. In the dog no nephrotomes were found anterior to somite 5, and it is now shown in the dog by a colleague that somite I-V comprise the occipital region rather than the cervical. As for tubule formation, the only pronephric tubules the dog forms are the ones with the quotes around them.

## The Mesonephros

In the dog, the mesonephrogenic tissue extends from somite 12-26 inclusive. The mesonephromeres began to differentiate after the nephric duct started growing caudad. This indicated some kind of interaction of an inductive nature between these tissues and suggested that the duct was behaving as an organizing

factor in mesonephromeric development as suggested by Torrey (1965) and Gier and Marion (1969). There are two criteria for the nephric duct to meet before it can be called a mesonephric duct: (1) it must be lumenated, and (2) mesonephric tubules must be attached to it. At this time the pronephric "tubules" are degenerating.

The works of Gruenwald (1937, 1942), Camber (1948), Burns (1938), Boyden (1927), Waddington (1938) and others, gave supportive evidence for the role of the nephric duct in the initiation and development of the mesonephros. Torrey (1965) pointed out that possibly there are other factors involved in this induction mechanism other than the nephric duct alone. In the dog, the pronephric "tubules" stimulate areas behind it to form more "tubules" which connect together to form the pronephric duct. Then back by somite 11-12, the tissue has become sophisticated enough to form mesonephromeres with the presence of the nephric duct.

The differentiation of the mesonephrogenic tissue started in the cranial end of the mesonephros and progressed step-wise caudal direction. Step 1 was the segmentation of the nephrotome into solid round mesonephromeres. The mesonephromeres slurred over an interesting phase of recapitulation by developing without a nephrostome. Step 2 showed the mesonephromeres organizing their cells so that at the end of this organization a central cavity appeared, surrounded by a layer of columnar cells, thus developing "cavitated mesonephromeres". Step 3, the cavitated mesonephromeres establish connections with the nephric duct via the proximal end of the sigmoid growing toward and attaching to the mesonephric duct rather than delamination as previously believed.

In the meantime the medial end of the cavitated mesonephromeres formed a

dorsal depression which expanded gradually until at the end there was a cup-shaped structure which was the anlage of the mesonephric (Bowman's) capsule. Step 4, the connecting portion began a flexion which initiated the S-shaped mesonephron. Early in the sigmoid stage, the two layers of the mesonephric capsule began to differentiate. The inner layer proliferated and thickened while the cells of the outer layer flattened. At the same time, some cells detached from the inner proliferated layer and entered the cavity of the vesicle. Altough it has been traditionally held that Bowman's capsule is established by invagination of the glomerular tuft into the wall of the mesonephromere (a view going back over 100 years to Remak), recent studies by electron microscopy (Kurz, 1958) indicate that invagination does not occur. Rather, the space between the ultimate filtering surface and the capsule arises as a cleft within a compact mass of epithelial cells, with the outer layer then becoming the capsular wall and the inner one reflected over the surface of the glomerulus. Vascularization of the mesonephric capsule started with the formation of the sigmoid tubule. A tuft of blood vessels, the glomerulus, was present inside the vesicle in this stage. Proliferation of the inner layer of the mesonephric capsule, and delamination of cells from it before the invasion of the segmental artery from the dorsal aorta, led to the suggestion that the mesonephric tubules participated in the formation of the glomeruli, and that the delaminated cells were of angioblastic potentiality and participated in glomerular formation (Martin, 1954). Observations in the dog gave no indication of this as mesonephric glomeruli were not observed to have pinched off from mesonephric blood vessels, but rather from long-tapered vasofactive cells which become organized into the vessels of the glomerulus. This lends support to the work done by Gier and Smith (1970) on the bovine heart. Seg-

mental arteries from the dorsal aorta to the glomeruli has been observed by most authors (DeMartino and Zamboni, 1966; Patten, 1968) to serve only one glomerulus per segmental artery. In the dog, one segmental artery serves 3 or 4 glomeruli (Figs. 23, 24).

Step 5, the connecting sigmoid portion continued to differentiate into a tubule of three distinguishable portions: the tubulus secretorious; tubulus intermedius, or ampullare; and tubulus collectivus. The tubulus secretorious was formed of columnar cells, while the tubulus collectivus was formed of cuboidal cells. The tubulus intermedius was a transitional area from the columnar type to the cuboidal type with two ampulla-like swellings thus giving it the name ampullare. This stage of the mesonephros was called the tripartite stage by Minot (1903). The relationship of the mesonephric duct to this form was different to that in the previous form. The mesonephric duct attained a position lateral to the mesonephros after having maintained a dorsolateral position in the previous forms. The glomerulus became medial to the rest of the mesonephros.

In step 6, the tripartite form developed a second sigmoid flexion involving the tubulus secretorious and tubulus intermedius. At the same time the tubulus intermedius developed a third ampulla thus becoming more complicated than the tripartite form, and developing the stage called the convoluted form. The glomerulus enlarged until it reached 100 micra or more in diameter. The combined expansion of the mesonephric tubule, glomerulus, and posterior cardinal vein bulged the mesonephros into the coelom.

At this point in development of the mesonephros, the phenomenon of transposterior cardinal vein migration in the dog occurs in the 35-36 somite stage. The mesonephric tubules directly traversed the lumen of the posterior cardinal vein from the mesonephric corpuscle to the mesonephric duct on the lateral side,

by elongation of the tubule and pressing of the tubule into the vein, as the vein pressed ventrally between the tubules. This condition was observed on several preparations of different embryos of this somite stage. As the basal portion of the posterior cardinal vein is cut off, it appeared to become the capillary network around the mesonephric tubules.

Most authors have explained the caudal movement of the mesonephros by degeneration of the anterior tubules while new ones form more caudad. This is not the case in the dog. In the embryos of 8 to 17 somites the anterior end of the mesonephros was at somite 12. In embryos of 34 to 36 somites, the anteriormost mesonephromeres were seen by somite 15. No picnosis or necrosis of cephalad mesonephric tubules were observed. This indicates a slippage of the anterior portion of the mesonephros because the posterior portion was more firmly attached and thus maintained its posterior position when the body growth exceeded mesonephric growth. Thus the phenomenon of posterior mesonephric movement is explainable in the dog by slippage rather than by degeneration.

The mesonephric duct differentiated in the dog from the intermediate mass of mesoderm. It made its appearance as a solid rod of cells, which at its caudal end laid very close to the ectoderm. Especially in its caudal portion, it was often contiguous with the somitic mesoderm. The material studied supplies no evidence for the assumption by other authors (Meyer, 1890; Felix, 1904; Hertwig, 1910), of a histogenetic participation of the ectoderm in the development of the primary excretory duct in mammals. The nephric duct elongates, root-like, and reaches the cloaca ventral to somite 28 in the 35-36 somite embryo.

All morphological and histological data indicated that the mesonephros

was functional in the dog embryo and played an important excretory role in its embryonic life. At 20 days the allantois begins to expand and fill with urine. At 21 days it reaches the serosa, and at 22 days, it is as big as the entire embryonic vesicle. In order for the allantois to expand it must be filled with urine from a functioning mesonephros.

## The Metanephros

The metanephrogenic tissue extended from the middle of somite 26 to somite 30 inclusive. In the 36-38 somite stage (22 day), it was a cone-shaped nephrogenic cord (Fig. 31). At the anterior level it was narrow and had a position medial to the nephric duct (Fig. 30). The mesonephric duct passes ventro-medially around the nephrogenic cord posterior to the end of the mesonephros, and presses against and becomes fused with the nephrogenic cord lateral to the groove between somites 28 and 29. As the pelvic region is differentiating and expanding rapidly at this time, tension on the mesonephric duct results in a pocket, the metanephric diverticulum, being pulled from the dorso-medial surface of the mesonephric duct where it adhered to the nephrogenic cord. The effective tension on the mesonephric duct is provided by pelvic elongation, which results in both elongation and separation of mesonephric and metanephric ducts until by 30 days, they are separate to the urogenital sinus. The final split is such that the orifice of the metanephric duct is lateral and ventral to that of the mesonephric duct. The sphinctor that characterizes the neck of the bladder develops between the orifices of the two sets of ducts. This is indication of interaction between the nephrogenic cord and the mesonephric duct leading to the origination of the metanephric diverticulum.

The metanephric diverticulum differentiates into two parts — a distal bulb-like portion which was the forerunner of the pelvis and a proximal tubular portion, which becomes the ureter. In this stage the metanephric blastema was a cap-like structure around the primitive pelvis. This pelvis then began to diverticulate sending primary diverticulae, which were the antecedents to the major calyces, and secondary ones, which are the antecedents of the minor calyces. The process of diverticulation continued until the final collecting tubules were formed. The blastema, developing around the diverticulating pelvis, differentiated into small masses which were the metanephromeres (Fig. 36). These went through the same essential steps through which the mesonephromeres had gone. They cavitated and formed the metanephric (Bowman's) capsule and connected with the tips of the collecting tubes by means of secondary outgrowths, with many capsules connecting to one tubule. The metanephric capsule had the same histological characteristics as the mesonephric capsule. Nephrogenetically, the metanephros or permanent kidney of the mature dog was of dual origin. The secretory elements were derived from the metanephrogenic tissue (nephrogenic cord) while the collecting elements were derived from the nephric duct.

During its development, the metanephros migrated from its place of origin ventral to somite 28 to a more anterior level, mainly by three processes: (1) the sacral flexure catches the early differentiating metanephric blastema within the bend and presses it anteriorly to a position anterior to the iliac artery and ventral to somite 26, (2) elongation of the mesonephros presses its anterior end anteriorly, as the posterior end is resting within the curve of the sacral flexure, anterior to the iliac artery, (3) straightening of the sacral flexure leaves the metanephros ventral to somites 26 to 28 inclusive.

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## EXPLANATION OF FIGURES

Fig. 1. Dorsal view of an 8 somite embryo (C1841) with the nephrotome (Nt) showing as a light line lateral to the somites on either side of the neural tube (NT) and the beginning of the pronephros (Pn) at somite 7 (S7). x10.

Fig. 2. Sagittal section of a 9 somite embryo (77L-3) with the nephrotome (Nt) lateral to somite 7 (S7) giving rise to the pronephros (Pn) dorsolaterally. x50.

Fig. 3. Transverse section of a 17 somite embryo (77L-1) with the first pronephric tubule (Pn) at the aspect of somite 7 (S7). x35.

Fig. 4. Transverse section of embryo 77L-1 with the pronephric duct (PD) over the pronephros (Pn) at somite 10. x35.

Fig. 5. Transverse section of embryo 77L-1 with a nephrostome (Ns) communicating with the coelom (C) at somite 8 (S8). x35.

Fig. 6. Transverse section of embryo 77L-1 with the pronephric "tubule" (Pn) on the left side at the back edge of somite 9 (S9), and nephrostome (Ns) on the right side. x35.

Fig. 7. Transverse section of embryo 77L-1 with the tip of the pronephric duct (PD) over the pronephros (Pn) at somite 11 (S11). x35.

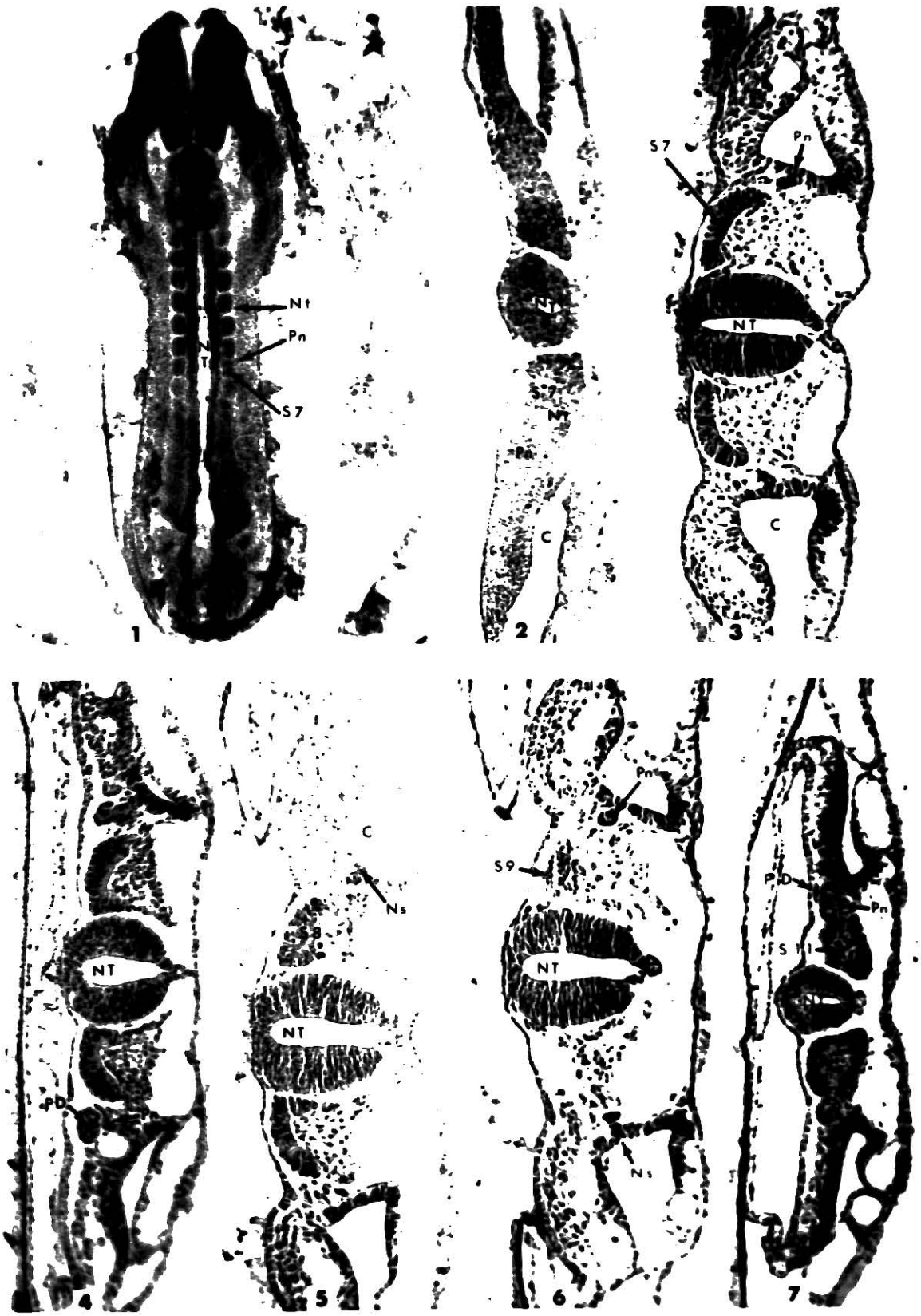

Nt
Pn
S7
NT
C
Ns
S9
PD
1
2
3
4
5
6
7

Fig. 8. Transverse section of a 17 somite embryo at somite 15 (S15) with the tip of the elongating nephric duct (Nd) over the mesonephric anlage (Ms). x35.

Fig. 9. Parasagittal section of a 25-26 somite embryo (45L) with pronephroi (Pn) at the front of somites 10 and 11. The pronephric duct (PD) over the pronephros (Pn) at somite 11 continues as the nephric duct (Nd) dorsal to the mesonephric anlage (Ms) from the front of somite 12 (S12) to the last somite. x35.

Fig. 10. Parasagittal section of embryo 45L with a pronephric remnant (Pn) anterior to the mesonephromeres (Ms). The mesonephric duct (Md) extends caudad over the differentiating mesonephromeres (Ms) as a solid cord. Yolk sac (YS) is in normal position. x15.

Fig. 11. Parasagittal section of a 35-36 somite embryo (254) showing the cavitated mesonephromeres (CMs) in relation to the somites (S). The amnion (Am) is complete at this stage. x20.

Fig. 12. A higher magnification of a 25-26 somite embryo (45L) with the nesonephric duct (Md) over the cavitated mesonephromeres (CMs). x50.

Fig. 13. Parasagittal section of a 35-36 somite embryo (254) to illustrate cavitated mesonephromeres (CMs) in numerical relation with the somites, particularly at somite 16 (S16). x50.

8
Z2
S15
Ms
Md
9
Pn
PD
Ms
S10
S12
10
Pn
Ms
YS
S
Ms
Md
11
CMs
S
Am
YS
12
YS
Md
CMs
13
CMs
S16

Fig. 14. Transverse section of a 47 somite embryo (241) with the anterior tip of the mesonephros (Ms) over the tip of the lung bud (LB). The mesonephric duct (Md) is ventral to the posterior cardinal vein (PCV), ventro-lateral to the mesonephric tubules (Ms), and dorso-lateral to the gut (G). x10.

Fig. 15. Transverse section of a 54 somite embryo (130RD) with the mesonephric tubule (Ms) extending across the posterior cardinal vein (PCV) over the posterior edge of the lung bud (LB). x20.

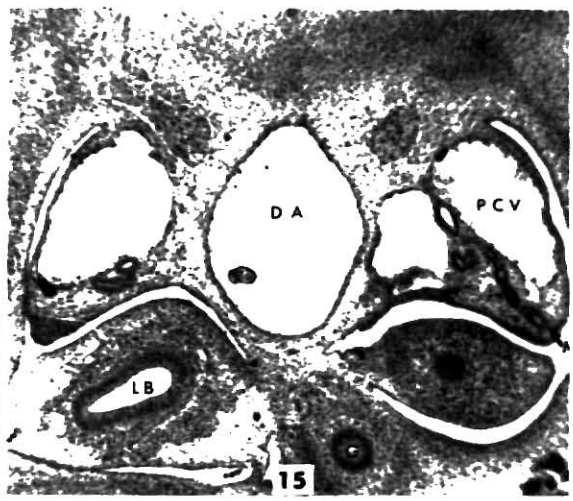
Fig. 16. Transverse section of embryo 241 with the mesonephric tubule (Ms) traversing the posterior cardinal vein (PCV) (left side). Vasofactive cells (VC) are differentiating to form the vessels of the glomerulus (right side). x20.

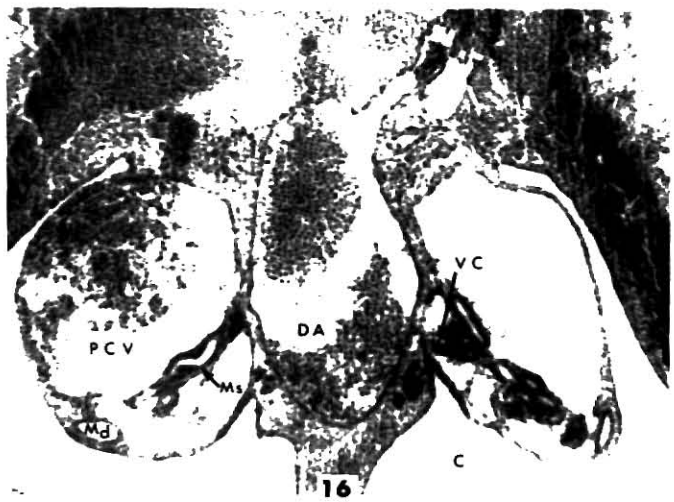
Fig. 17. Transverse section of a 35-36 somite embryo (81Rd) with the mesonephric tubule (Ms) crossing the posterior cardinal vein (PCV) and connecting laterally to the mesonephric duct (Md). The posterior cardinal vein (PCV) is separated from the subcardinal vein below the mesonephros (Ms). This section shows a portion of the neural tube (NT) and the anterior end of the liver (L). x20.

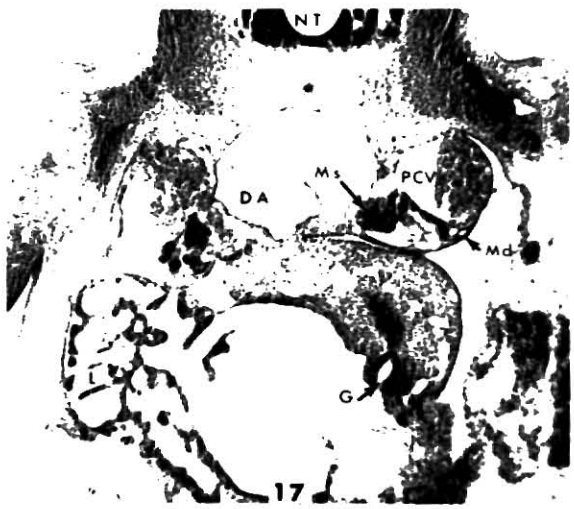
Fig. 18. Transverse section of a 34-35 somite embryo (49L) with the anterior end of the mesonephros (Ms) in step 4 of mesonephron differentiation. The mesonephron is in the sigmoid condition. x50.

Fig. 19. Transverse section of a 36 somite embryo (D354R) with segmental arterioles (SA) from the dorsal aorta (DA) supplying the glomeruli (Gl) of the mesonephromeres (Ms). x20.


PCV
DA
Ms
LB
Md
G
14


DA
PCV
LB
G
15


VC
DA
PCV
Ms
Md
C
16


NT
Ms
PCV
DA
Md
G
17


PCV
DA
Md
Ms
18

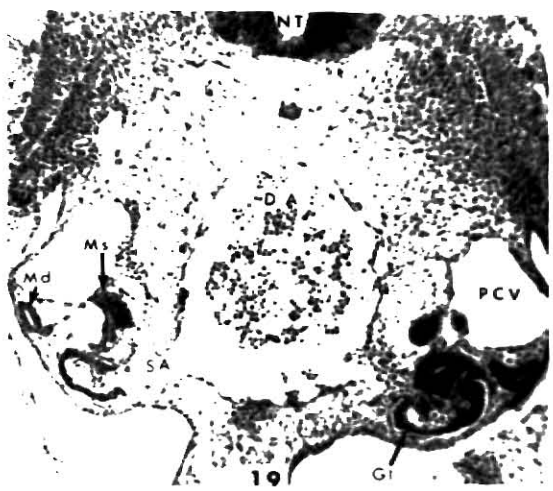

NT
D
Ms
Md
PCV
SA
19

Fig. 20. Transverse section of a 54 somite embryo (130RD) with the mesonephromeres (Ms) over the stomach (G) and liver (L). A single segmental artery (SA) connects from the dorsal aorta (DA) to a glomerulus (Gl) within a mesonephric capsule. x20.

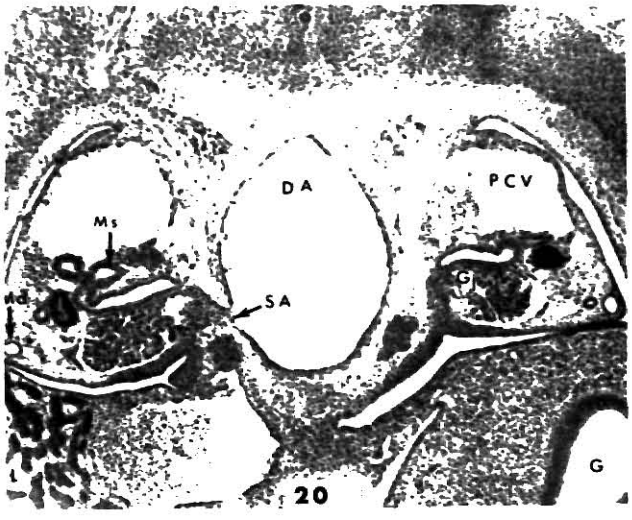
Fig. 21. Transverse section of a 36 somite embryo (48R) showing relationships of the glomerulus (Gl) to the mesonephric tubule (Ms) and mesonephric duct (Md). x20.

Fig. 22. Transverse section of a 30 somite embryo (D354R) with the mesonephric tubule (Ms) attached to the mesonephric duct (Md) thus separating the posterior cardinal vein (PCV) from the subcardinal vein (SV). x20.

Fig. 23. Oblique transverse section of a 54 somite embryo (130RD) at the vitelline artery. A segmental artery (SA) from the dorsal aorta (DA) supplies more than one glomerulus (Gl). The subcardinal vein (SCV) is separated by mesonephric tubules from the posterior cardinal vein (PCV). The gonadal ridge (Gd) hangs below the mesonephros. x20.

Fig. 24. Parasagittal section of a 50 somite embryo (C953) showing one segmental artery (SA) supplying 5 glomeruli (Gl). x60.

Fig. 25. Frontal-transverse section of a 54 somite embryo (130RD) showing the junction of the mesonephric duct (Md) and metanephric duct (MD) medial to the umbilical artery (UA) and ventral to the mesonephros (Ms). x10.



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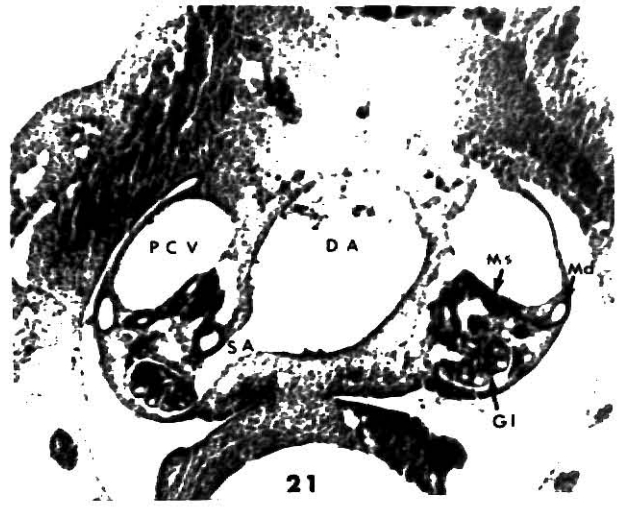

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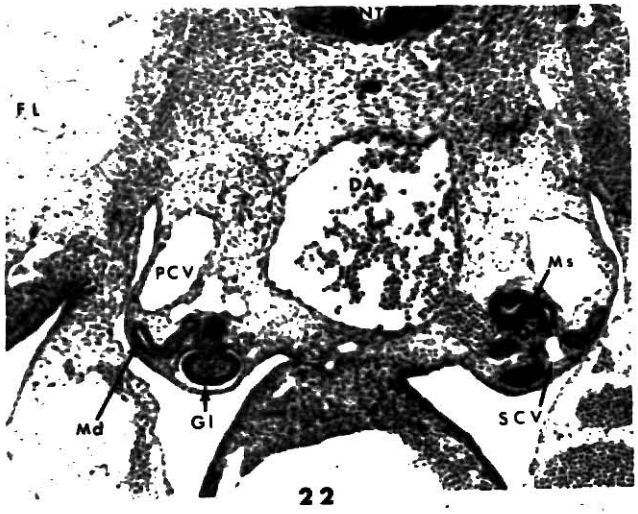

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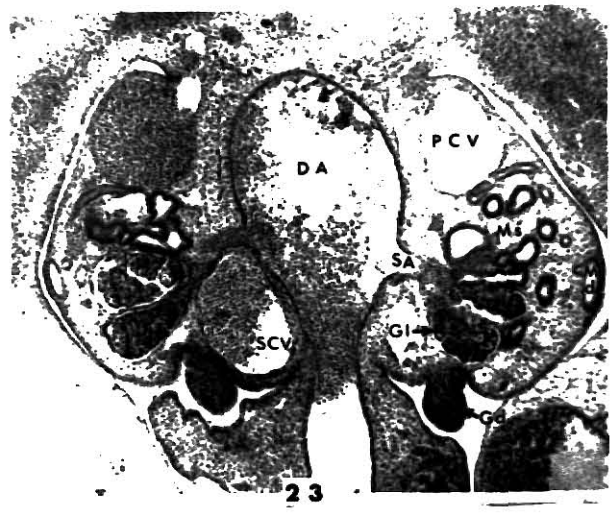

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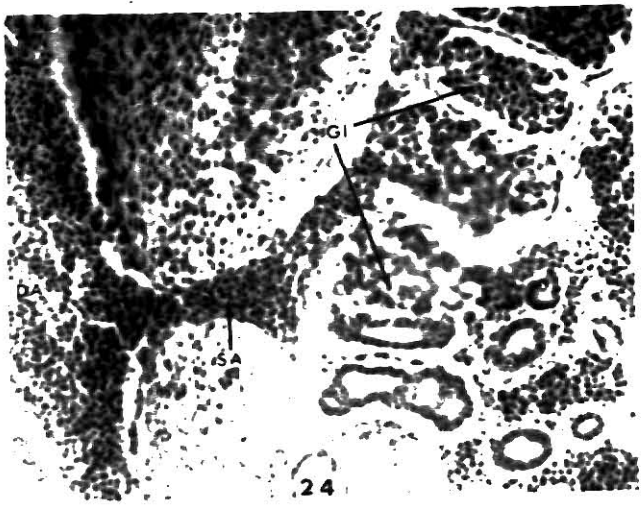

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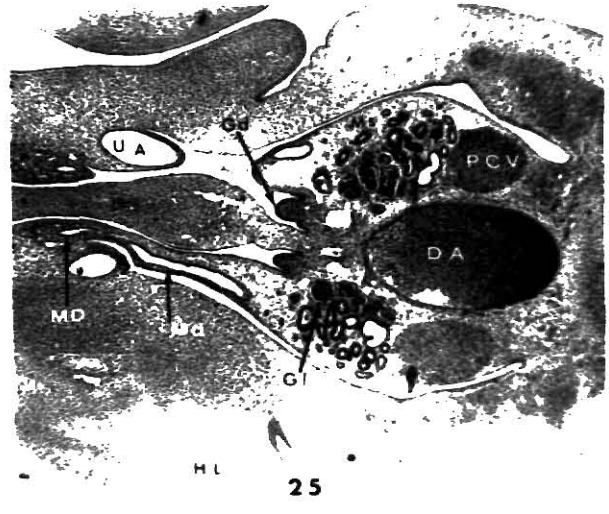

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Fig. 26. Parasagittal section of a 35-36 somite embryo (D358LA) with the mesonephros (Ms) extended from somite 12 (S12) to somite 26 (S26). Glomeruli (Gl) are well developed and due to the cut of the section and torsion of the posterior part of the embryo, the mesonephric duct (Md) can be seen only between somites 21 and 24. The mesonephric duct (Md) is broadly connected to the urogenital sinus (UGS). x15.

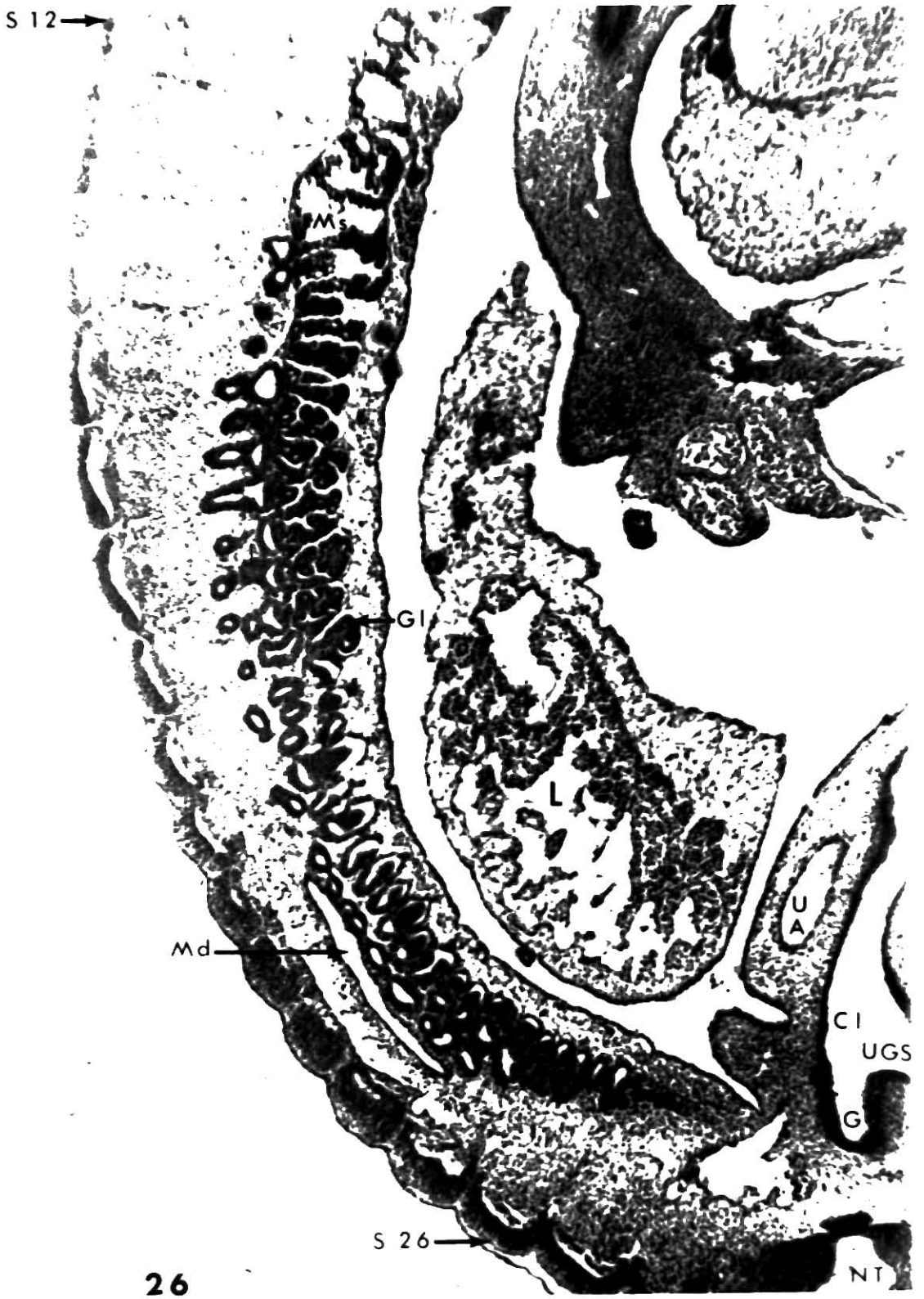


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Fig. 27. Transverse-frontal section of a 54 somite embryo (130RD) with the mesonephric duct (Md) entering into the urogenital sinus (UGS). The mesonephros (Ms) and gonadal ridge (Gd) are dorsal to the umbilical arteries (UA) in the hind limb bud (HL) region. x20.

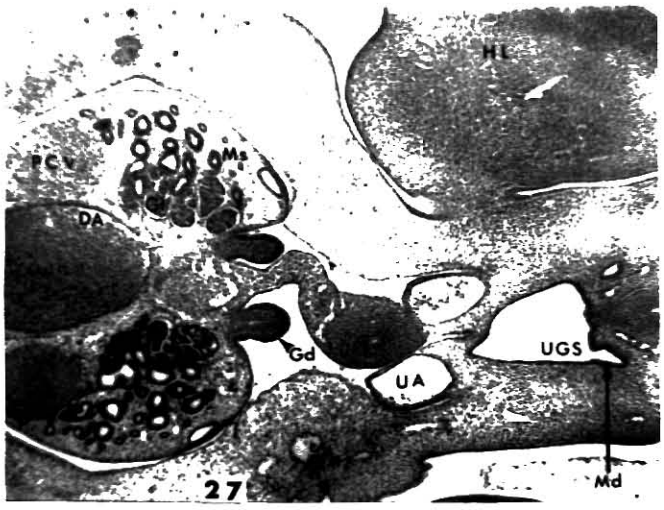
Fig. 28. Oblique-transverse section through the cloacal region (Cl) of a 28 somite embryo (42L) at the point where the posterior tip of the mesonephric duct (Md) connects with the urogenital sinus (UGS). x50.

Fig. 29. Oblique-transverse section through the cloacal region and somites 26-27 of a 36 somite embryo (D354R) with the mesonephric duct (Md) entering the urogenital sinus (UGS). The dorsal aorta (DA) is double in the caudal region of the embryo with the mesonephros (Ms) and nephrogenic cord (NC) ventro-lateral to it. x20.

Fig. 30. Oblique-frontal section of the sacral flexure region of embryo D354R where the mesonephric duct (Md) has moved ventral and medially around the nephrogenic cord (NC) making a metanephric anlage in which the metanephric blastema develops from the nephrogenic cord (NC) and the metanephric pelvis develops from the mesonephric duct (Md). x20.

Fig. 31. Oblique-transverse section of embryo D354R anterior to the point of contact between the nephrogenic cord (NC) and the mesonephric duct (Md). x20.

Fig. 32. Diagonal section through somite 27 of a 35 somite embryo (49L) where the nephrogenic cord (NC) is in active contact with the mesonephric duct. The metanephric blastema (MB) and the metanephric diverticulum are differentiating from this point of contact. x50.



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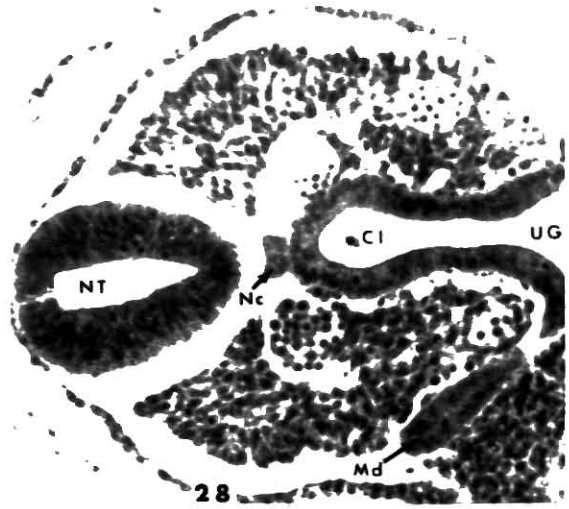

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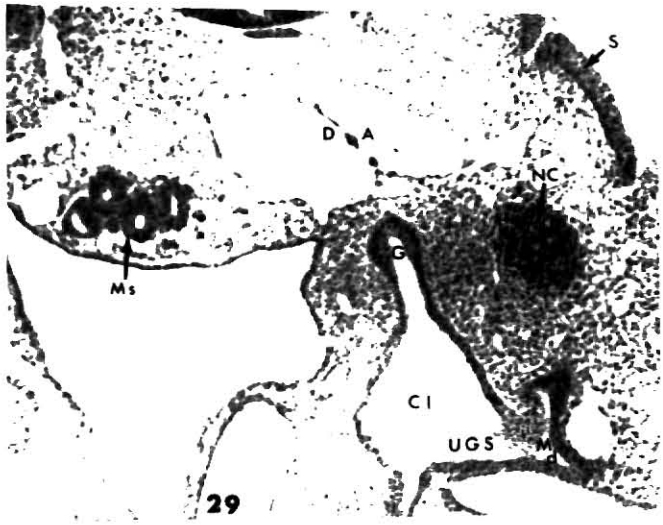

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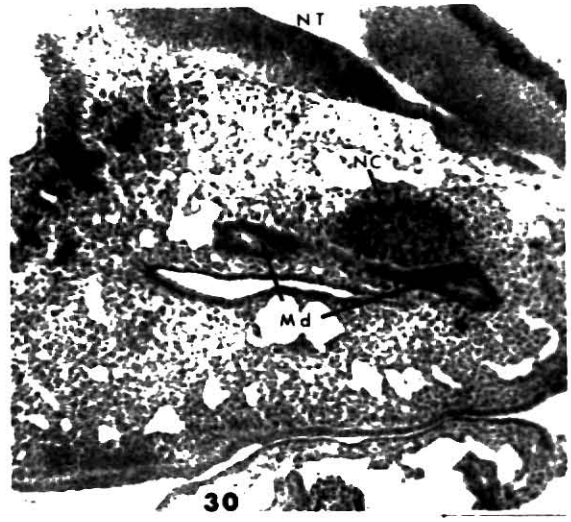

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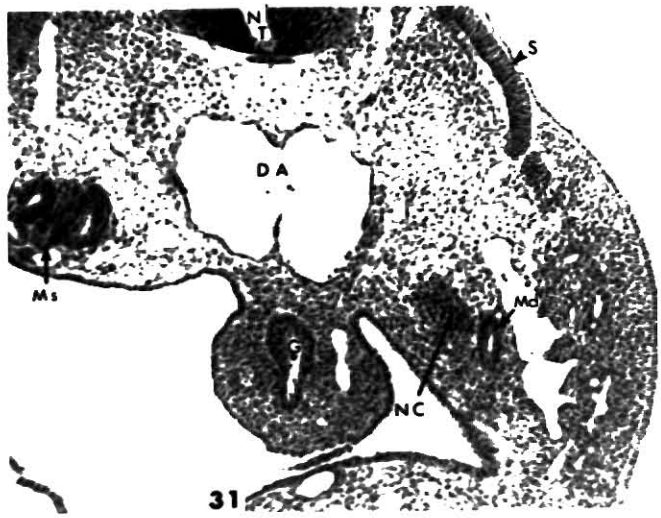

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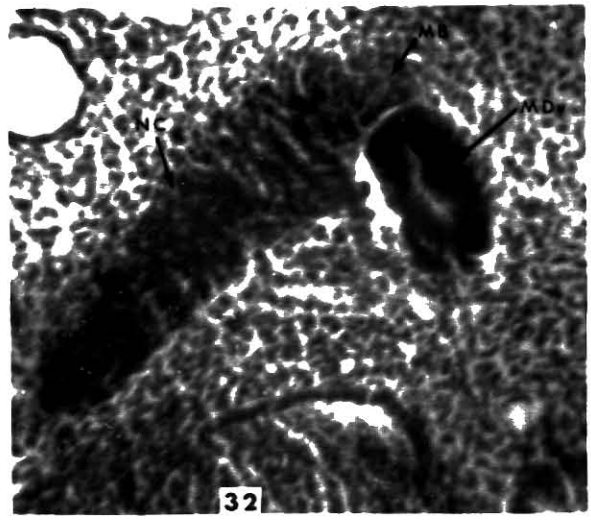

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Fig. 33. Oblique-transverse section of a 36 somite embryo (D354R) with the metanephric pelvis (MP) and metanephric blastema (MB) undergoing differentiation at the point where the mesonephric duct (Md) and nephrogenic cord (NC) come together ventro-lateral to the dorsal aorta (DA) and lateral to the urogenital sinus (UGS). x20.

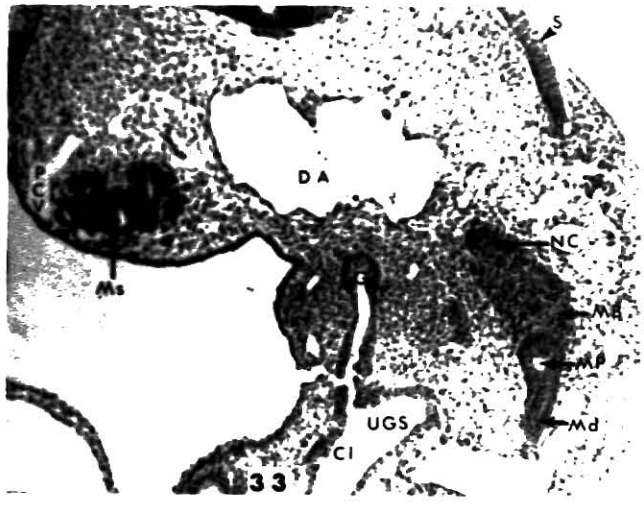
Fig. 34. Parasagittal section of 45 somite embryo (123RC) with metanephric blastema (MB) and metanephric pelvis (MP) forming lateral and posterior to the iliac artery (IA). The old nephrogenic cord remains as the gubernaculum (Gb). The common genital duct connects the metanephric duct from the metanephric pelvis (MP) with the urogenital sinus (UGS). x20.

Fig. 35. Oblique-transverse section through the hind gut region of a 36 somite embryo with the gut (G) and urogenital sinus (UGS) medial to the differentiating metanephric blastema (MB) and metanephric pelvis (MP). The mesonephric duct (MD) is ventral to the metanephric pelvis (MP) and lateral to the gut (G).

Fig. 36. Oblique-transverse frontal section of a 54 somite embryo (130RD) with the developing metanephros (Mt) and the metanephric duct (MD) medial to the iliac artery (IA), medio-ventral to the posterior cardinal vein (PCV), ventro-lateral to the dorsal aorta (DA) and posterio-dorsal to the mesonephros (Ms). x20.

Fig. 37. Transverse section through the metanephros of a 50 somite embryo (C953) where the metanephric duct (MD) is medial to the differentiating metanephric blastema (MB) which almost encloses the metanephric pelvis (MP). x75.

Fig. 38. Transverse section of a 48 somite embryo (145LC) in the hind limb bud region with the developing metanephroi (Mt) posterio-dorsal to the last mesonephric structure (Ms). x30.



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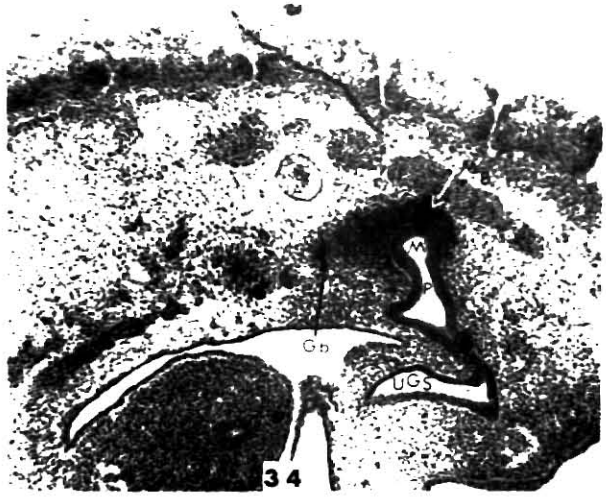

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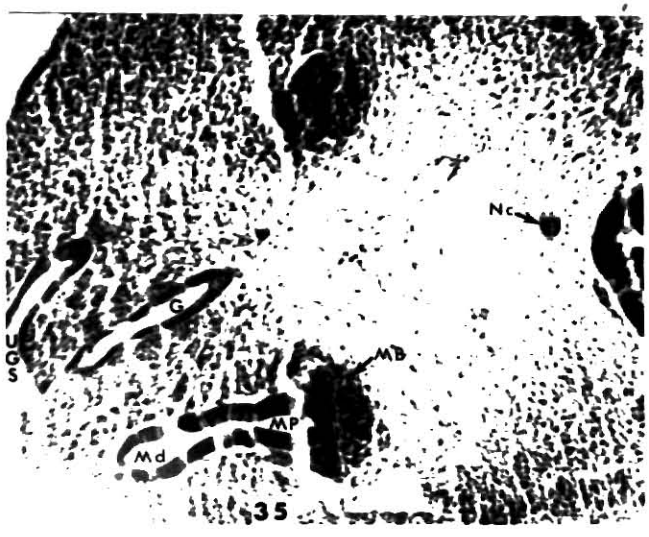

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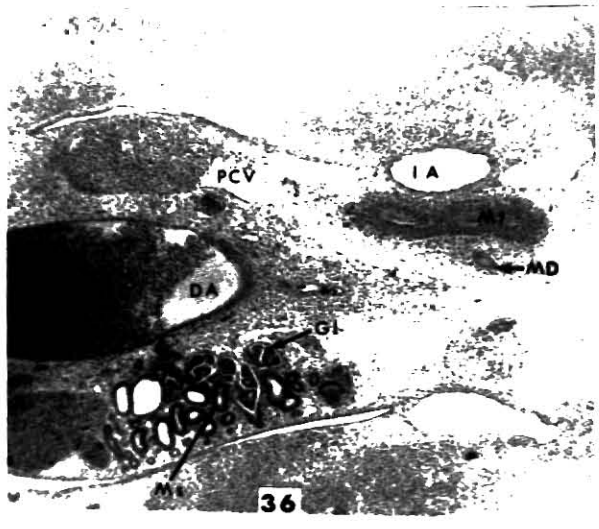

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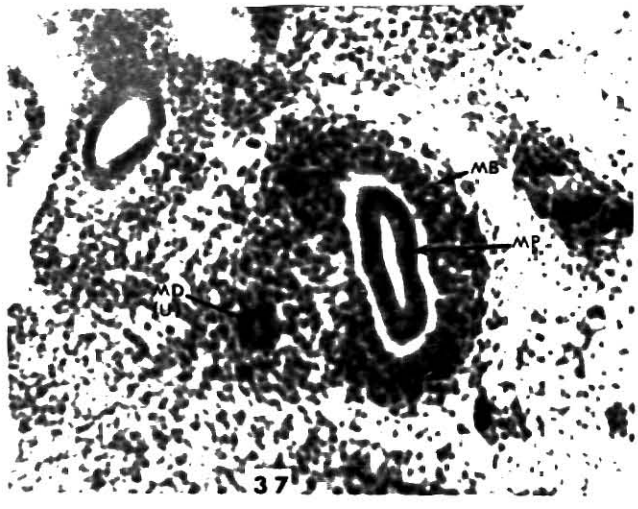

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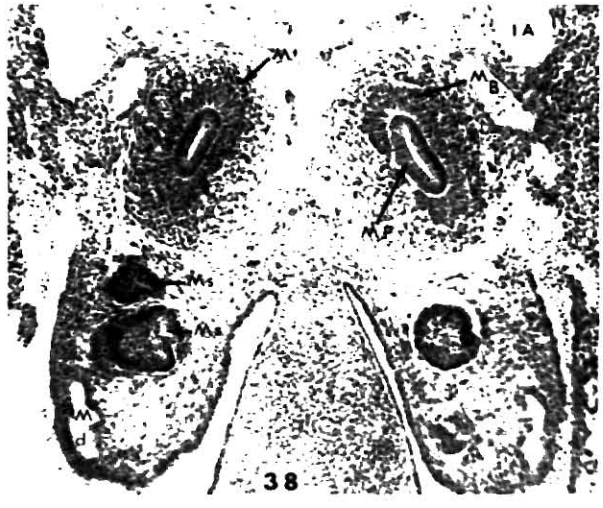

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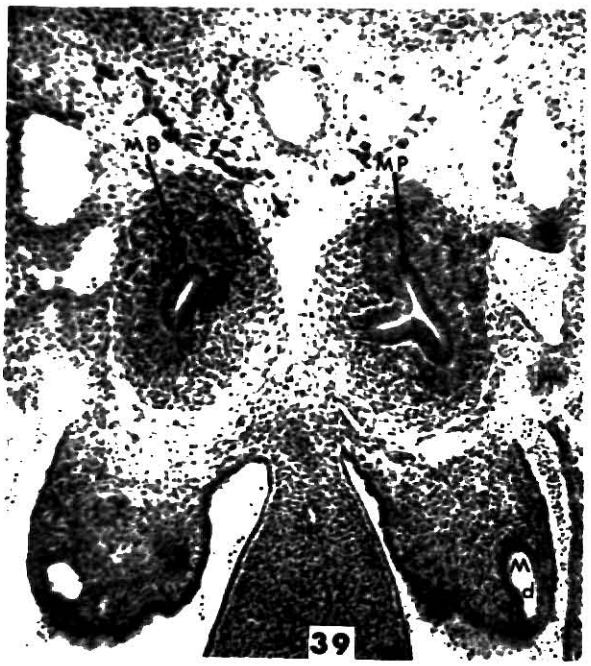
Fig. 39. A cross section of embryo 145LC further caudad, posterior to the mesonephros, but through the mesonephric ducts (Md) before it reaches the cloaca. The metanephric pelvis (MP) on the left completely encloses the metanephric pelvis in this section but on the right a little more posteriorly, the blastema is incomplete where the ureter (U) is continuous with the metanephric pelvis. x35.

Fig. 40. A longitudinal section of the metanephros of a 54 somite, 26 day embryo (46L) with the metanephric pelvis (MP) undergoing secondary diverticulation to form the major calyces of the kidney. The metanephric blastema (MB) surrounds these secondary diverticulations. x35.

Fig. 41. A longitudinal section of the kidneys of a 54 somite, 27 day embryo (60R) with the metanephric pelvis (MP) diverticulating into tertiary branches. The metanephric blastema (MB) has subdivided into masses around each branch. x20.

Fig. 42. A longitudinal section of a 54 somite, 30 day embryo (9L) with the parts of the metanephros in definitive relationships. Metanephric corpuscles with classic glomeruli (Gl) and in Bowman's capsules (BC) have formed. The metanephric pelvis (MP) has diverticulated into its major calyces (Cx) which in turn are subdividing into minor calyces surrounded by the blastema comprising the renal cortex (RC). The permanent metanephros is developing dorsal to the testis (T) and dorso-lateral to the liver (L). x35.



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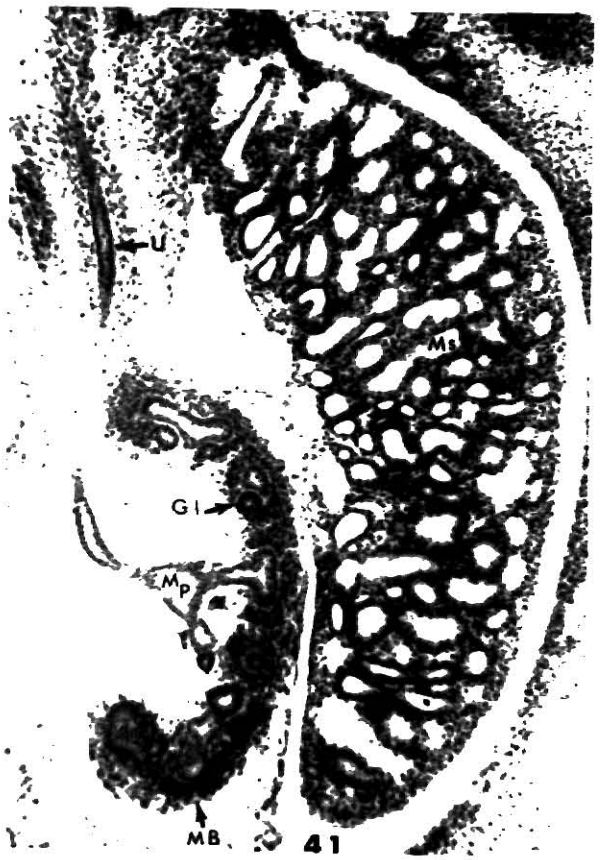

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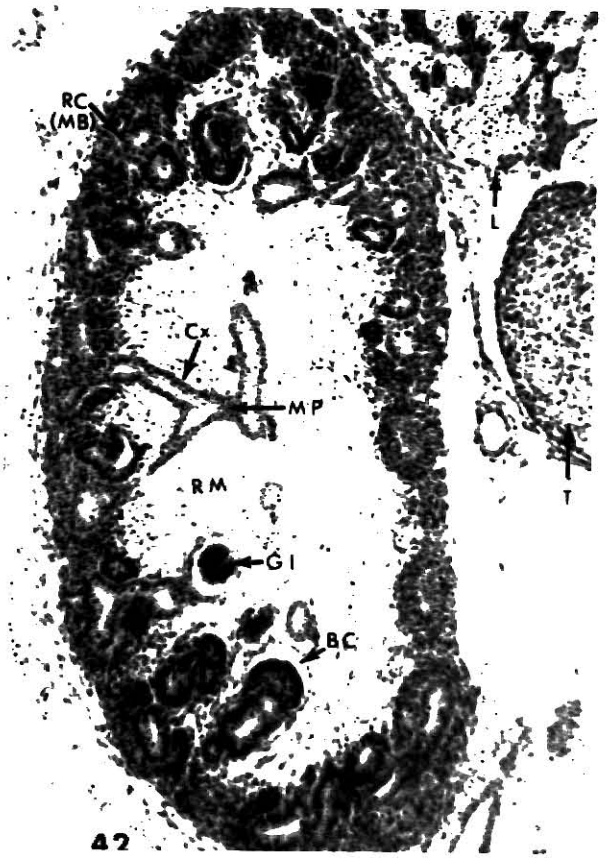

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# NEPHROGENESIS IN MAMMALS

by

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B. S., Kansas State University, 1969

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## ABSTRACT

Nephrogenesis has been adequately described in only a few mammals. Because of the small number of mammals that have been studied, the conflicts in interpretations, and the need for a comprehensive, detailed study of nephrogenesis in other mammals, a study of the processes in the dog was made possible by the accumulation of a large number of serially sectioned canine embryos dated as to somite number and days gestation. Over 30 embryos were examined in detail, and 20 others checked for various points. This study is concerned with nephrogenesis, from the first showing of pronephric differentiation from the nephrotome to the establishment of the metanephros.

The pronephric tissue extends from somites 7 through 11; the nephric duct reaches the cloacal region by posterior growth of the pronephric duct when the embryo is in the 25-26 somite stage, and is fully lumenated at the 35 somite stage. The mesonephrogenic tissue extends from somites 12 through 26; the mesonephromeres start to differentiate shortly after the nephric duct extends caudally over the nephrotome posterior to somite 12; mesonephromeres appear as solid spheres of cells within the nephrotome with three per somite lateral to somite 12 and increasing to four per somite at somite 22. Mesonephromeres lumenate progressively from somite 12, posteriorly, then elongate into a sigmoid structure which connects to the adjoining mesonephric duct. Mesonephric corpuscles develop by expansion of the medial end of the sigmoid, and the proximal end nearest the mesonephric duct elongates as the mesonephric tubule. Glomeruli form in the mesonephric corpuscles by organization of vasofactive cells and each segmental artery from the dorsal aorta supplies 3-5 glomeruli.

The metanephrogenic cord extends from somite 26 through 30; the nephric duct, in its caudal growth, crossed ventro-medially to the nephrogenic cord, resulting in mutual contact and interaction between the two, thus establishing the metanephric anlage. Tension on the duct by posterior elongation of the pelvis causes elongation of the metanephric diverticulum as the mesonephric duct is pulled away from the nephrogenic cord. The associated nephrogenic cord is stimulated by that association to differentiate into metanephric blastema which in sequence differentiates metanephromeres, then nephric tubules and Bowman's capsules, with glomeruli. The metanephric anlage originates ventro-laterally to somite 28. By a combination of forces from sacral flexure, pelvic expansion, and elongation, the metanephros is shifted slightly anteriorly until it lies ventral to somite 26-28 inclusive.