CANAINE RENAL DEVELOPMENT AND REVERSIBLE IRRADIATION DAMAGE IN THE MESONEPHRIC AND METANEPHRIC KIDNEYS

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ABSTRACT

CANINE RENAL DEVELOPMENT AND REVERSIBLE IRRADIATION DAMAGE IN THE MESONEPHRIC AND METANEPHRIC KIDNEYS

The development of the mammalian kidney occurs through three embryologically related stages: pronephros, mesonephros and metanephros. Each stage contributes structural components to the succeeding stage. The early renal stages regress and are replaced by the most efficient permanent metanephric kidney. The regression of the intermediate and first functional mesonephric kidney takes place with simultaneous development and maturation of the metanephros.

The maturation of the canine nephrogenesis continues at least through the second week of postnatal life. This process was thought to be responsible for the vulnerability of this organ to a variety of insults; among these is the irradiation effect to which the developing kidney is more sensitive than is adult kidney. The radiosensitivity of the developing kidney is associated with the presence of an active peripheral nephrogenic zone in the postnatally maturing kidney.

Animals exposed to 100 R at different stages of gestation and sacrificed at 7 days postirradiation reveal a detectable interference with nephrogenesis in both metanephric and mesonephric kidneys. However, with elongated postirradiation period in animals designated for delayed effect of irradiation and sacrificed at 55 days of gestation, no significant morphologic or morphometric changes were observed when
compared with the controls. This, of course, does not eliminate the
radiosensitivity of the actively dividing nephrogenic cells, at any
stage but with irradiation at early nephrogenesis sufficient time
exists for continued nephrogenesis and the acquisition of a relatively
normal metanephric kidney.

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TABLE OF CONTENTS

1. Introduction

Materials and Methods

2. Results

Normal Embryological Development of the Beagle Kidney

Development and differentiation of the Mesonephros

Period I (21 dpc - 25 dpc)

Period II (26 dpc - 28 dpc)

Period III (32 dpc - 42 dpc)

Development and differentiation of the Metanephros

Period I (22 dpc - 25 dpc)

Period II (25 dpc - 28 dpc)

Period III (32 dpc - 57 dpc)

3. Effect of Radiation Upon the Developing Beagle Kidney

Immediate Effect of Irradiation

Group I (Irradiation at 15 dpc - sacrifice at 22 dpc)

Group II (Irradiation at 21 dpc - sacrifice at 28 dpc)

Group III (Irradiation at 28 dpc - sacrifice at 35 dpc)

Group IV (Irradiation at 35 dpc - sacrifice at 42 dpc)

Delayed Effect of Irradiation

4. Discussion

5. Literature Cited
FIGURE LEGENDS

Fig. 1. Cross section of 21-25 dpc control fetus. The mesonephros is located lateral to the dorsal aorta which supplies branches to the nephrons of the mesonephros. X95

Fig. 2. Sagittal section of the early mesonephros of 21-25 dpc control fetus. Individual nephrons are arranged in a single row. Each of the anterior glomeruli, on the right, has a single medially located mesonephric tubule. X38

Fig. 3. Mesonephros of 21-25 dpc control fetus. Differentiated cranial nephrons are seen at the left while immature corporules identifiable by their poorly developed capillary networks are seen at the right. X95

Fig. 4. The entire mesonephros of a 21-25 dpc control fetus. Well differentiated cranial nephrons are at the right, while immature nephrons are located at the upper left. X38

Fig. 5. A normal fully developed mesonephros at 26-28 dpc. In its posterior portion, the nephrons exhibit a high level of development and possess long well differentiated tubules. X38

Fig. 6. A normal mesonephros of the 26-28 dpc group. Early regression of the anterior region is seen where the number of nephrons is markedly reduced. X95

Fig. 7. A normal mesonephros of 26-28 dpc group reveals shrinkage and disorganization of the corporules. A small nondifferentiating corporule lined by cuboidal parietal epithelium and enclosing a shrunken glomerular tuft is seen in the middle of the field. X380

Fig. 8. Advanced regression of the anterior mesonephros in a control fetus of 26-28 dpc group. This process is characterized by thickening of the glomerular tuft and is accompanied by necrosis and the accumulation of cellular debris in Bowman's space. X242

Fig. 9. Control embryo from the 26-28 dpc group. The liver appears as a large lobulated structure composed of numerous hepatic cords. The stomach represents a tubular structure in the middle of the field. The mesonephros, gonad and metanephros are seen on the upper right. X38

Fig. 10. A control mesonephros of 32-42 dpc group. The anterior portion contains few markedly regressing glomeruli embedded in a dense connective tissue layer. Large mesonephric glomeruli surrounded by numerous tubules are seen in the posterior portion. Dilatation of the tubules is frequent in this region. X61
Fig. 19. A metanephros from a fetus approximately 57 days of gestation. The nephrogenic zone is still active. The immature nephrons are composed of S-shaped structures and renal vesicles and are found in the outer cortex and overlying a thin metanephric blastema region. The underlying successive nephron stages are located in the inner cortex separated by radiating medullary rays. The oldest and most well developed nephrons are seen in the cortico-medullary juncture at the bottom. X60

Fig. 20. A mesonephros from a fetus irradiated at 15 dpc and sacrificed at 22 of gestation. The nephrons are arranged in a single row as in the control in Fig. 1. However, the majority of these nephrons possess corpuscles with poorly differentiated capillary networks. X38

Fig. 21. A sagittal section of the mesonephros of a fetus irradiated at 21 dpc and sacrificed at 28 dpc. The posterior portion of the mesonephros at the left contains immature corpuscles which possess thick glomerular tufts. (Compare with the control mesonephros in Fig. 5 where the posterior mesonephros is fully developed.) X38

Fig. 22. A mesonephros of a fetus irradiated at 21 dpc and sacrificed at 28 dpc demonstrating cranio-caudal regression comparable to the control in Fig. 6. X35

Fig. 23. Posterior mesonephros at the left and early branch of the ureteral bud at right from a fetus irradiated at 21 dpc and sacrificed at 28 dpc. (Compare with Figs. 15 and 16 which demonstrate the advance metanephroneogenesis expressed by multiple renal vesicles and S-shaped nephrons in a control fetus of comparable gestational age.) X152

Fig. 24. Anterior regressing and posterior differentiating segments of the mesonephros with metanephros situated dorsomedially. This fetus was irradiated at 21 dpc and sacrificed at 28 dpc and represents the only animal from this group in which both kidneys resembled those of the controls. X38

Fig. 25a,b,c,d The outer nephrogenic zone from the metanephros of animals irradiated at 28 dpc and sacrificed at 35 dpc. The changes in this group are characterized by reduced activity in the nephrogenic zone. The main feature here is the decreased blastema cell density seen as loose areas (c,d). Also the ampullary branches are in close proximity to the capillary surface and are capped by a thin metanephric blastema cell layer and associated with few renal vesicles (d). (Compare with control of similar age in Fig. 17.) Magnification in all is X243 except c where magnification is X680.

vii
Fig. 26. A metanephros from a fetus irradiated at 35 dpc and sacrificed at 42 dpc. The nephrogenic zone is thin and exhibits decreased formation of new nephrons (compare with control of comparable age in Fig. 17 where more active nephrogenic zone is observed). X152

Fig. 27. A kidney from a fetus at 28 dpc and sacrificed at 55 dpc. A slightly thin, less active nephrogenic zone is seen in comparison to the control in Fig. 16. X61

Fig. 28. A kidney from an animal irradiated at 35 dpc and sacrificed at 55 dpc. The nephrogenic zone is less active and a large number of S-shaped nephrons are located beneath the capsule. Different immature nephron stages are seen in the inner cortex associated with poorly developed medullary rays. (Compare with the control, Fig. 16.) X61
Fig. 13. Early metanephric development of 22-25 dpc fetus is initiated by protrusion of the ureteral bud from the mesonephric duct and its interaction with the metanephric blastema. The latter appears as cellular clusters arranged around the ureteral bud. X239

Fig. 14. A normal early metanephros of the 25-28 dpc group. The terminal portions of the ureteral bud, the ampullae, undergo dichotomous branching while invading the metanephric blastema. An early renal vesicle is seen lateral to the ampulla on the right. X243

Fig. 15. An early S-shaped nephron in the metanephros of the 25-28 dpc group. A renal vesicle is seen at the right. X306

Fig. 16. From the same group as Fig. 15; multiple S-shaped structures are seen in the outer cortex. The medulla is composed of a loosely arranged connective tissue stroma and contains few tubular structures. X242

Fig. 17. A well developed kidney of an animal of the 32-57 dpc group. The outer cortex at the periphery is composed of numerous, densely packed immature nephrons, while the inner cortex possesses primarily the oldest mature nephrons recognizable by their prominent Bowman's space lined by flat, columnal epithelial layer. At this stage the overall area of the cortex appears to be larger than the small mesenchymal cell medulla. X38

Fig. 18. A metanephros from a fetus approximately 42 dpc. A well delineated superficial nephrogenic layer composed of numerous immature nephron stages is seen in the outer cortex at the top. Deep to this layer are nephrons in various stages of maturation with the most mature being located closest to the corticomedullary function. In the deep cortex corpuscles are surrounded by developing tubules. The medulla at the bottom is sparsely populated by tubules which consist primarily of collecting ducts.
INTRODUCTION

An understanding of the embryological development of the kidney is an important prerequisite for the understanding of the normal and pathological nephron. There is wide variation in the rate of renal anatomical and functional maturity from one species to another. In the human fetuses, nephrogenesis is complete by the 35th week of gestation so that at term the full number of nephrons are present (34,44). In contrast, nephrogenesis in the dog continues at least through the second week of postnatal life (12,23). The lamb and guinea pig appear to fall somewhere in between the human and canine neonates (34).

In vertebrates, embryological development of the kidney proceeds through successive well-defined stages which include: the rudimentary, short-lived pronephros; the mesonephros which gives rise to the ureteral bud and, in turn, through interaction with the metanephric blastema, induces formation of the definitive kidney, the metanephros (15). These three stages share a common origin. They arise from the lateral plate known as the intermediate cell mass which gives rise to the nephrotome which later differentiates into the successive renal stages.

The first full descriptive report dealing with these renal stages comes from Felix (13) in which he describes them as separate anatomical entities succeeding one another in time. However, in the majority of cases, the pronephros is an undetectable structure except in those animals with larval stages (15). In the human, the pronephros degenerates
at the same rate with which it is formed (44).

The degenerating pronephros is succeeded by the well differentiated mesonephros, which is considered to be the first primitive functional unit of the urinary system. It consists of a tubular system which connects the renal corpuscles to the mesonephric duct. The tubular segment adjacent to the glomeruli is called proximal and that portion leading to the mesonephric duct is known as distal. The proximal tubules are differentiated from the distal by their thick wall, brush border and their strongly eosinophilic cytoplasm (7,35,49,52).

One cannot overemphasize the importance of the pronephros and mesonephros for the development of the definitive kidney. There is general agreement that each nephron stage contributes structural components necessary for the development of the successive stages (15, 34,44). It has been shown that the pronephric duct of the degenerating pronephros extends caudally and continues as the mesonephric duct (15). Similarly, Potter (44) and Nash (34) have shown that the ureteral bud, which emerges from the mesonephric duct, must interact with the metanephric blastema to induce the development of the metanephros.

The dual origin of the metanephros is now well accepted. Its differentiation begins when the ureteral bud, originating from the mesonephric duct, begins to grow in a dorsocephalic direction and invades the metanephric blastema of the nephrogenic cord. Wessells (53) has suggested that both components may produce chemical substances which interact and induce the differentiation of the definitive kidney.

The differentiation of the human metanephros was studied by Osathanondh and Potter (36-39) using the technique of microdissection. They concluded that the ureteral bud divides dichotomously and forms
tubules which dilate and later differentiate into the renal pelvis, calyces and collecting tubules. The terminal portions of the ureteral bud, the ampullae, come into contact with the isolated mass of the metanephric blastema. As the ampulla branches and elongates, the undifferentiated blastemal cells advance with the ampulla, resulting in the induction of nephrons and stromal connective tissue. The continuous addition of nephrons contributes to the growth and maturation of the metanephros.

Several investigators (15,27,32) have demonstrated that mammalian kidney in different species exhibits a functional overlapping between the mesonephros and metanephros; especially in late gestation. This idea was also expressed by Gersh (16), who used phenol red as an indicator for tubular function in pregnant rabbits and found that the embryonic mesonephros functions to eliminate the dye and continues to do so until the metanephros gradually and finally assumes the excretory function.

McCance's (32) experiments have demonstrated that mammals such as the pig and sheep possess a well developed mesonephros that is functionally active until the metanephros gradually and finally assumes excretory function in late fetal life, while in the rabbit, guinea pig, man, and rat generation of the mesonephros takes place before the metanephros has matured. The importance of the mesonephros as a functional organ has been further stressed by Stainer (51). On the basis of these findings, many investigators agree upon the overlapping function of the mesonephros and metanephros, especially in late gestation.

In the canine, maturation of the metanephros continues into the postnatal period which makes this organ sensitive for a longer period to a variety of insults, among which is the irradiation effect to which
the developing kidney is more sensitive than adult kidneys. This sensitivity was attributed to the presence of an active nephrogenic zone in the outer cortex (10,19,20). Research in this field has demonstrated a high incidence of severe progressive glomerulosclerosis associated with a high incidence of renal failure in perinatally irradiated dogs (10,25,26,30,41). In experiments in mice, Guttmann and Kohn (22) have observed an increase in the incidence of spontaneous progressive glomerulosclerosis following exposures to irradiation shortly before or after birth as compared with exposures at 12, 27, or 53 days of age.

Dose-dependent kidney lesions are also reported in the literature (21,22,41). Phemister et al. (41) have noted that low levels of whole-body irradiation to young dogs produces more severe lesions in comparison with those produced with the same dose applied during adult life. In other experiments Guttmann and Kohn (22) have observed that mice receiving a single dose of 250 R at 0 day of age had equivalent kidney lesion when 500 R was applied in 12-day-old mice. It was concluded that the developing kidney is more sensitive to relatively small exposures of irradiation than mature kidneys.

The immediate effect of irradiation was studied by Guttmann and Kohn (21) where newborn mice received 475 R and were examined at 12 hours and 4 days postirradiation. Changes attributed to irradiation were identified as degenerative changes in the primitive epithelial cells of the Bowman's capsule accompanied by reduction in the nephrogenic zone and glomerular malformation. Similar results were obtained by Eisenbrandt and Phemister (10,11) using 2-day-old dogs and 330 R exposures. In these animals as early as 6 hours of postirradiation
produced an extensive necrosis in the S-shaped nephrons, ampullae and renal vesicles. Both arrested and dysplastic corpuscles were also seen.

Investigation of the delayed effects of irradiation demonstrated an increased amount of intercellular membrane-like material in the vascular pole of the glomeruli. Other changes were characterized as glomeruli with shrunken glomerular tufts, necrotic debris, mineralization, distention of the Bowman's capsule and occasional fibrosis (10,22). In other studies in beagles, irradiated at 55 days in utero or 2 days of age, there was a thinning of the renal cortex and a decrease in the density of the mature and total glomeruli. With age, the severity of the lesions progresses and are characterized as mesangial sclerosis and chronic renal failure (11,12,19,20,22,26,41). Therefore, dose-at-exposure and age-at-exposure are both considered as determining factors.

The purpose of this report is to study the immediate and delayed effect of irradiation in the developing kidney where the animals were irradiated in utero at different ages. A second objective is to trace the normal development of the canine kidney and compare it with those of the irradiated groups.
MATERIALS AND METHODS

The animals used in this study were obtained from a closed beagle colony involved in a long term investigation of the effects of in utero and early postnatal irradiation at the Collaborative Radiological Health Laboratory, Fort Collins, Colorado. The animals in this study were divided into three major groups on the basis of their treatment. The first group included 78 fetuses obtained from pregnant bitches during the 21st to 57th days of gestation. These fetuses were utilized for study of the normal development of mesonephric and metanephric kidneys and correlation of the development of other organs. The second group of fetuses was utilized in the study of the immediate effect of irradiation to the kidney; these fetuses were divided into four subgroups which were irradiated at different stages of gestation and sacrificed at 7 days of postirradiation. Fetuses in the last group were also irradiated in utero but were designated for the study of the delayed effect of irradiation on the developing kidney. Four subgroups, irradiated at different in utero stages, were all sacrificed at 55 dpc. Table 1 shows the distribution of the animals according to their treatment.

On the preselected gestation day, bitches in the irradiated groups received a single bilateral exposure to cobalt 60 gamma radiation for a constant time of 10 minutes resulting in a total dose of 150 R. Exposures were limited to the abdomen by lead shielding over the head.
<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>No. of animals in each group</th>
<th>Age at irradiation</th>
<th>Days between irradiation and sacrifice</th>
<th>Tissue</th>
<th>Dose</th>
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<td>Delayed effect</td>
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* This is a control group.
neck, thorax and limbs. All exposures were recorded at mid-line air
dose to the animals. Matching control animals were sham-irradiated.

Embryos and fetuses from all groups were surgically removed and
the kidneys or entire fetus fixed in 10% buffered formalin or Bouin's
fixatives. Serial sections were prepared from paraffin-embedded blocks,
stained with hemotoxylin and eosin or PAS and examined by light
microscopy.

In the last group, where the delayed effect of irradiation was
studied, further morphometric methods were used to evaluate the nephro-
ogenic activity in the cortex. Eight to nine animals from each subgroup
and an equal number of controls were randomly selected and one kidney
section from each animal was divided into four or five representative
sampling locations of the cortex. The perimeter of the outlined areas
was measured in mm using a videoplan device (Karl Zeiss, Inc. 1980).
Different renal stages which included renal vesicles, S-shaped forms,
immature and mature glomeruli located in the sampling areas were
counted. The number of nephron stages per unit area was interpreted as
the density and calculated by the following formula:

\[
density = \frac{\text{number of nephron components}}{\text{area in mm}^2}
\]
RESULTS

Normal Embryologic Development of the Beagle Kidney

For descriptive purposes the mesonephric and metanephric kidneys are presented separately, however, it is important to recognize that these two components undergo simultaneous growth and maturation and that regression of the former occurs in parallel with development of the later. The sequential development of each kidney is presented in three arbitrary time periods, however, the rate with which this process occurs varies between fetuses as well as between litters.

Development and Differentiation of the Mesonephros

Period I (21 dpc - 25 dpc). In cross sections of the embryo, the mesonephros is located lateral to the dorsal aorta which supplies the nephrons with mesonephric arteries. Lateral to the mesonephros is located the mesonephric duct which is a tubular structure lined by cuboidal epithelial cells and which serves as the excretory duct of the mesonephric tubules (Fig. 1).

In sagittal sections, the organ appears as single nephrons spaced at regular intervals along the long axis of the mesonephric duct (Fig. 2). At the beginning of this period an early craniocaudal differentiation of the mesonephros is seen. This process begins as simple renal vesicles which, by a process of folding and indentation, form S-shaped structures which finally differentiate into functional glomeruli. The most rostral one-third of the mesonephros possesses corpuscles whose
capsules are lined by flat epithelium and contain glomerular tufts with moderately dilated capillaries. The less differentiated nephrons are located caudally and possess densely cellular corpuscles with few patent capillaries (Fig. 3). Both the visceral and parietal layers of these corpuscles are lined by closely packed cuboidal epithelial cells.

The mesonephric tubules also appear to follow the same pattern of cranio-caudal differentiation. The tubules of the cranial, more mature nephrons, are composed of two relatively distinct components, i.e., proximal and distal tubules. The proximal tubules have thicker walls, smaller lamina and abundant eosinophilic cytoplasm with basally located nuclei. In contrast, the distal tubules have thinner walls, wider lumina and a small amount of basophilic cytoplasm with uniformly round, centrally located nuclei. The caudal portion of the organ, whose nephrons are less mature, contains relatively undifferentiated tubules associated with a small number of immature developing corpuscles (Fig. 4). At this stage, specific structures identifiable as the promesonephros are not observed.

This period of early mesonephric development is accompanied by the appearance of the optic vesicles and initial development of early gonads from the genital ridge. The latter appears as a solid mass of cells protruding ventromedial to the mesonephros. The liver has a small number of hepatic cords separated by dilated blood-filled sinusoids. The heart appears as a thin walled structure that seems to be divided into two main chambers (atrium and ventricle).

Period II (26 dpc - 28 dpc). At this stage, the mesonephros appears to be at the height of its maturation. Its middle and caudal portions are slightly thicker than the shrunken anterior portion (Fig. 5).
The thickening of the posterior region is attributed to the presence of a large number of differentiated tubules which are especially abundant in this region. The majority of the nephrons in the middle and posterior segments are fully matured except for a few in the most caudal segment. Both the parietal and visceral epithelial cells of the differentiated corpuscles are squamous and contain a small amount of cytoplasm. In some embryos a few large corpuscles with markedly lobulated glomerular tufts are present in the posterior portions of the mesonephros. Further nephron formation appears to have ceased at this stage.

As maturation of the mesonephros proceeds, evidence of degeneration and regression, associated with a reduction in the number of nephrons (Fig. 6), beginning in the rostral one-third of the organ, becomes noticeable. Small numbers of shrunken tubules and even fewer mesonephric corpuscles are seen scattered in a proliferating connective tissue stroma. The corpuscles in this region are smaller in size and are difficult to distinguish from the adjacent tubules. This is manifested by a dedifferentiated parietal epithelium. The glomerular tuft, which undergoes a marked reduction in size, has a small number of collapsed capillaries which are covered by a cuboidal layer of closely packed visceral epithelium (Fig. 7). An additional contributing component to the regressive changes, occurring within the anterior region, is the occasional necrosis of the parietal epithelium. This layer contains small numbers of disorganized cells whose cytoplasm is shrunken and granular with no recognizable outline. The nuclei are deeply hyperchromatic with occasional karyorrhexis. Small amounts of cellular debris are seen within Bowman’s spaces of the affected corpuscles (Fig. 8).
Both proximal and distal tubules located in the anterior mesonephros are shrunken and widely separated by the proliferating connective tissue stroma. The epithelium of these tubules has a vacuolated to frothy, usually acidophilic cytoplasm with an irregular apical surface. Their nuclei are darker and no longer maintain their basal or central location, but are swollen and frequently appear karyolytic.

At this stage, the liver has a distinct lobular pattern and is composed of a large number of hepatic cords which contributes to the large size of the organ (Fig. 9). The division of the heart into separate chambers is evident; the left ventricular wall is much thicker than in the previous stage. All cardiac chambers are filled with a varying amount of blood. The pancreas is now visible as clusters of acini scattered within a loose connective tissue stroma lying in close proximity to the liver and duodenum. The rib cage is well developed and encloses the thoracic cavity. The later is separated from the abdominal cavity by a thin diaphragm. Late in this period, the lungs appear as multilobulated structures that consist of branching bronchiolar components; however, no alveolar structures are readily recognizable. The eye has a pigmented retinal epithelium separated from the lens by a sensory layer.

**Period III (32 dpc - 42 dpc).** This period is characterized by a further regression in the anterior mesonephros. The anterior portion is now much thinner and shorter than the large club-shaped and highly differentiated posterior portion. While the anterior mesonephros is observed to undergo active regression consisting of varying degrees of necrosis and desquamation of mesonephric components, the caudal portion exhibits a growth and complexity greater than that observed in
the previous stages (Fig. 10). The posterior nephrons are composed of large corpuscles whose glomerular tufts appear to be multilobulated and possess numerous widely dilated capillaries. These corpuscles are associated with a large number of tubules that contribute to the characteristic appearance of this region and serves as an indication of the high level of mesonephric functional maturity apparently required at this stage of embryonic life. At this level of differentiation and complexity a third mesonephric tubular component is recognized. In contrast to the proximal and distal tubules, the newly recognized tubular segment has cuboidal epithelium with scant basophilic cytoplasm; these have been identified by both Novikov (35) and Tiedemann (52) as collecting tubules.

In sagittal sections the gonads and developing metanephros appear as oval structures located dorsal and ventral to the anteriorly regressing mesonephros (Fig. 11). At this stage the liver appears larger than in previous stages. Its hepatic cords are more numerous and well differentiated and the sinusoids are compressed by the expanding hepatic parenchyma. Nucleated red blood cells and a variable number of megakaryocytes are frequently seen in the sinusoids. The gallbladder is identified in association with the ventral hepatic lobe.

The pericardial sac is well developed and separates the heart from other adjacent organs. A distinct communication of the heart with the lungs and liver by large vessels (i.e., pulmonary arteries, veins and vena cava) is obvious. The lungs consist of three distinct lobes: apical, cardiac and diaphragmatic and exhibit extensive branching of the bronchi and bronchioles. The pulmonary alveoli are lined by loosely arranged epithelial cells with indistinct cytoplasmic borders (Fig. 12).
The gastro-intestinal tract is represented by a large number of cross-sectioned tubular structures indicating an increase in length and tortuosity. The cornea of the eye is a convex structure covering the anterior portion of the globe. The vitreous is well developed while the retina appears as a darkly pigmented layer of cells.

**Development and Differentiation of the Metanephros**

**Period I (22 dpc - 25 dpc).** The earliest evidence of metanephrogenesis occurs when the tubular ureteral bud, located dorsomedial to the caudal portion of the mesonephros, advances dorsally to invade clusters of metanephric blastema. The latter are arranged as multilayered aggregates of basophilic pyramidal cells (Fig. 13).

**Period II (25 dpc - 28 dpc).** This period is characterized by branching of the ureteral bud which gives rise initially to structures which will eventually form the renal calyces. Continued peripheral advancement and dichotomous branching results in the formation of tubular ampullae which invade the peripheral mass of metanephric blastema. This interaction results in the formation of oval masses of cells located at the angle between the ampullae and collecting ducts (Fig. 14). These cells further differentiate into renal vesicles whose lining columnar epithelial cells radiate towards a central lumen. At this point the medulla appears as a centrally located mass of loose connective tissue within which are embedded a small number of collecting ducts.

Further dichotomous branching of the ampullae and their continued interaction with the blastema cells result in the formation of an additional successive population of vesicles in the subcapsular region. Simultaneously the earlier generation of metanephric vesicles is
displaced to a deeper layer of the cortex where it is observed to undergo further differentiation into S-shaped forms (Figs. 15,16). The lower indentation of the S-shaped form appears to consist of two distinct lamina lined by columnar cells and separated from each other by a thin layer of mesenchymal cells. The latter probably serves as a vehicle through which capillaries gain access to the corpuscles. The Bowman's capsule of the S-shaped form which is continuous with the collecting ducts is thicker and lined by columnar cells. The remainder of the wall is lined by cuboidal cells which become progressively thinner and more distended. Further differentiation of these layers gives rise to the visceral and parietal epithelium of the corpuscles.

Period III (32 dpc - 57 dpc). As a result of continuous growth and maturation the metanephros is now approximately half as large as the regressing mesonephros. Its cortex is well delineated from the medulla and peripherally is covered by a hypercellular nephrogenic zone which contains a large number of renal vesicles and S-shaped forms. Located in the deep cortex are the first generations of metanephric corpuscles which appear as small basophilic structures composed of densely packed, poorly vascularized glomerular tufts. As maturation progresses these inner cortical glomeruli become larger and less densely cellular. Their capsules are lined by squamous parietal epithelium which encloses well vascularized glomerular tufts which resemble those seen in adult kidneys (Figs. 17 and 18).

This distributional pattern of the metanephric components permits an arbitrary division of the organ into four, morphologically distinct zones. The first zone is identified as the outer cortex and is composed of a thin subcapsular metanephric blastemal cell region overlying
branches of ampullae and their associated renal vesicles. The second zone contains collecting ducts extending inward from the ampullae and a large number of scattered S-shaped structures and immature glomeruli. Zone three, located deeper in the cortex, includes a variable number of mature glomeruli evolving from the peripherally located immature nephrons. The fourth zone of the metanephros is identified as the medulla and contains small clusters of basophilic tubules surrounded by a relatively dense mesenchymal layer.

Glomerular differentiation in this period is accompanied by differentiation and maturation of the metanephric tubules. In contrast to the previous period where small number of undifferentiated tubules predominated, this period of metanephric development is characterized by the presence of a large number of tubules which exhibit evidence of morphologic differentiation into distinct functional segments, i.e., proximal, distal and collecting tubules. The proximal tubules have more eosinophilic cytoplasm and are more abundant in the outer cortex. The distal segments are lined by lower cuboidal cells with less eosinophilic cytoplasm. The collecting tubules are found in the cortex and more frequently in the medulla and are lined by cells with a small amount of basophilic cytoplasm and round nuclei. This period is also associated with the appearance of medullary rays consisting of a large number of peripherally radiating collecting ducts recognizable by their deep basophilic appearance (Fig. 19).

At the end of this period, which coincides with late gestation and preparturition, the kidney is still an actively developing organ with a prominent peripheral nephrogenic zone. This activity is associated with appositional growth of the organ and successive maturation of the nephrons.
EFFECT OF RADIATION UPON THE DEVELOPING BEAGLE KIDNEY

Immediate Effects of Irradiation

Group I (Irradiation at 15 dpc - sacrifice at 22 dpc).

The immediate effects of irradiation were examined in four groups of animals irradiated at successive stages of gestation and fetal development followed by collection of fetal kidneys seven days postirradiation.

The morphology of the normal kidney at this age is described above and is characterized by the craniocaudal differentiation of mesonephric nephrons and branching of the ureteral bud from the mesonephric duct which extends dorsally to invade clusters of metanephric blastema.

The mesonephros of the irradiated fetuses of this group exhibits less advanced craniocaudal differentiation in comparison to nonirradiated animals. In the former group only a few nephrons appear to exhibit differentiation levels similar to the mesonephros of control animals (Fig. 20). The delayed mesonephric maturity in the irradiated animals is even more noticeable in the posterior portion where the majority of the nephrons appear as immature or S-shaped nephrons.

Tubular differentiation is also less conspicuous in irradiated fetuses than in controls where the mesonephric tubules are undergoing initial differentiation. In exposed fetuses the tubules do not show a recognizable differentiation pattern; instead they are deeply basophilic and lined by small, uniformly round cells. Moreover, the initial formation of the ureteral bud from the mesonephric duct as seen at this age in control animals is not observed in the irradiated group.

Group II (Irradiation at 21 dpc - sacrifice at 28 dpc). During this period the mesonephros of control animals exhibits continued posterior maturation and anterior regression accompanied by annular
branching in the metanephros with the formation of renal vesicles and S-shaped nephrons.

The mesonephros of the irradiated animals may be divided into two subgroups on the basis of their maturity. The first subgroup represents embryos which exhibit the least degree of maturity and in which the mesonephros is still undergoing early development. The normally expected advanced differentiation level of the mesonephros is expressed only in the most anterior portion of the organ while the posterior one-third contains predominantly immature nephrons (Fig. 21).

Kidneys of the second subgroup of irradiated fetuses are more mature and exhibit a degree of differentiation approximately equal to that of control kidneys (Fig. 22). The anterior regression of the mesonephros observed in the control kidneys is present though less prominent in exposed fetuses, while the posterior mesonephros possesses well differentiated nephrons similar to those seen in control animals.

In the majority of the irradiated fetuses examined, the metanephros consists primarily of early branches of the ureteral bud and terminal ampullae around which are located aggregates of metanephric blastema (Fig. 23). In only one animal of this group it was possible to recognize a well delineated metanephric kidney comparable to those seen in controls (Fig. 24). This kidney was characterized by a distinct, though thin nephrogenic zone accompanied by renal vesicles and evolving S-shaped nephrons.

Group III (irradiation at 28 dpc - sacrifice at 35 dpc). In this period renal development in nonirradiated animals characterized by extensive regression in the anterior mesonephros while the posterior segment possesses numerous fully mature complex nephrons. The metanephros
also exhibits advanced maturation and possesses a well differentiated cortex and medulla.

The mesonephros of irradiated animals is shorter and more globoid than in the previous stage, however, anterior regression and posterior maturation are less advanced than in sham-irradiated controls. The irradiated metanephric kidney, like that of controls, is also composed of a distinct cortex and medulla, however, the nephrogenic zone is thinner and the blastema cell zone appears to be less densely populated than in controls (Figs. 25a,b,c). While in controls the ampullae are capped by layers several cells thick, the ampullary branches in irradiated fetuses are located in direct association with the capsule and have relatively thin metanephric blastema cell caps of disorganized cells with dark nuclei (Fig. 25d). Few renal vesicles are seen accompanying these ampullae; instead, varying numbers of S-shaped nephrons are seen in the surrounding areas. The inner cortex is predominantly composed of immature nephrons amount which only a few mature glomeruli are observed.

The number of the mesonephric tubules is also reduced in these areas as compared with the controls; however, the tubules appear to be properly differentiated into proximal, distal and collecting segments. In kidneys of the irradiated group, the medulla appears approximately twice as large as the cortex and contains only a few scattered basophilic tubules.

**Group IV** (Irradiation at 35 dpc - sacrifice at 42 dpc). The anterior portion of the mesonephros of sham-irradiated animals appears as a thin, elongated segment containing a large number of regressing nephron accompanied by numerous multilobulated well developed corpuscles
in the posterior region. The metanephros of these animals is seen to be undergoing advanced appositional growth of new nephrons.

If irradiated animals mesonephric regression is active and involves the entire anterior portion, while the posterior segment is populated by large mesonephric corpuscles with multilobulated occasionally disorganized glomerular tufts. Mesonephric tubules which exhibit varying degrees of dilatation are scattered among these corpuscles.

The metanephros of irradiated animals is seen to be undergoing progressive maturity, however, the nephrogenic zone is focally disorganized and reduced in width when compared with the kidneys of the controls. Areas of reduced blastema cell thickness associated with thin nephrogenic zone which were observed in the previous group are more prominent in these animals (Fig. 26; compare with Fig. 17).

Tubular dilatation in the outer cortex is also seen in the kidneys of irradiated animals as well as in the control, but is more frequent in the irradiated kidneys.

Delayed effect of irradiation

The delayed effect of irradiation was also examined using four groups of animals irradiated sequentially in utero but all sacrificed at 55 dpc.

The kidneys of the sham-irradiated dogs sacrificed at 55 dpc reveal a prominent, active nephrogenic zone overlying numerous nephron generations of successively greater maturation level (Fig. 10). Kidneys of animals irradiated at 15, 21, 28 and 35 days in utero and sacrificed at 55 days do not differ significantly from those of the controls.

This is more clear in animals irradiated at 15 and 28 dpc. However, a slight thinning of the nephrogenic zone is seen in some individuals
irradiated at 28 (Fig. 27) and at 35 dpc (Fig. 28). In these two
groups there is also a reduction in the size of medullary rays.

Morphometric analysis of the density of renal vesicles, S-shaped
forms, immature and mature glomeruli showed no significant differences
between the metanephric kidneys of control and irradiated fetuses at
55 dpc (Table 2).
Table 2. Evaluation of the nephrogenic activity.

<table>
<thead>
<tr>
<th>Age in days</th>
<th>No. of animals</th>
<th>Mean of vesicles &amp; s-shaped</th>
<th>Standard error</th>
<th>Mean of immature glomeruli</th>
<th>Standard error</th>
<th>Mean of mature glomeruli</th>
<th>Standard error</th>
<th>P</th>
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<td>10.53</td>
<td>± .72</td>
<td>8.18</td>
<td>± .59</td>
<td>.81</td>
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<td>10.80</td>
<td>± 1.05</td>
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<td>± .69</td>
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<td>± .44</td>
<td>8.48</td>
<td>± .66</td>
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</table>

* P value is counted as a comparison between the control and irradiated animals.
DISCUSSION

The development of the urinary system demonstrates morphological similarities between the mesonephros and metanephros. Both systems begin as renal vesicles which then progress to S-shaped tubular structures and finally into mature nephrons composed of capillary tufts enclosed in a capsule and tubules which are differentiated into distinct structural and functional segments (15,43,48). However, these systems also exhibit prominent dissimilarities. As shown in the present study, the mesonephros is an elongated structure composed of individual nephrons which mature in a cranio-caudal direction. On the other hand, the metanephros is an oval structure which develops centrifugally by a process of superficial nephrogenesis. While the mesonephros undergoes simultaneous cranial regression and caudal differentiation, the metanephros is involved in a continuous process of peripheral expansion by the addition of new nephrons to the outermost cortex and the continuous maturation without regression of the earlier developed deep cortical nephrons.

Although cranio-caudal regression of the mesonephros has been described by a number of authors (4,8,27,28,47) there is little agreement regarding the mechanism responsible for this process. In this study, metanephric regression involved dedifferentiation of the corpuscles which was characterized by shrinkage of the glomerular tuft with accumulation of dense material in the glomerular vascular pole and finally
necrosis and fragmentation. The mesonephric tubules in the regressing region showed marked collapse and progressive cytoplasmic vacuolation proceeding their complete disappearance. DeMartino et al. (8) in electron microscopic studies demonstrated similar changes in the human mesonephros. They attributed these changes to mesangial hypertrophy with concomitant deposition of electron-dense mesangial matrix which was believed to be responsible for the vascular obliteration of the glomeruli. They compared this process with nephropathies of adult kidneys characterized by mesangial proliferation leading to glomerulosclerosis. Other investigators have suggested that mesonephric regression is due to ischemic changes resulting from a shift in the local circulation from the indirect portal type to the adult type (8). Morris (33), who examined the regressing chick mesonephros noted a marked decrease in mesonephric size and weight which he attributed to vasoconstriction and infiltration of the regressing region by connective tissue. Thyroid hormone, which reaches its peak activity during the time of regression, is also thought to be involved. Other investigators have speculated that this hormone also plays a significant role in amphibian pronephric necrosis and possibly in the mesonephric regression as well (8,14). However, its precise mechanism of action in these processes is unknown. Phagocytic cells were not seen in the mesonephroi examined in this study which agrees with previous investigator's (4,8, 14,33) observations in other species. It appears that different factors may operate separately and simultaneously in the process of regression, however, the most likely one is the vascular component. In the regressing nephrons the glomerular tuft was shrunken and the capillaries were few and collapsed. This idea was suggested by
DeMartino et al. (8) who noted that the progressive obliteration of the glomerular vascular bed was brought about by mesangial hypertrophy resulting in the deposition of amorphous material and collagen fibrils along the wall of the glomerular capillaries.

The mesonephros, which is considered to be the first functional mammalian kidney, shares this function with the definitive metanephric kidney in late gestation (27,28). While the anterior mesonephros undergoes regional regression, its posterior segment exhibits continuous growth and differentiation. The growth of the mesonephros is accompanied by simultaneous growth and development of the early metanephric corpuscles which undergo progressive maturation. The simultaneous presence of morphologically mature corpuscles in both kidneys suggests overlapping function of both organs at this particular time.

The relationship of the mesonephros to the metanephros is not only functional but also embryological (2,15,34,43,44,53). The definitive kidney begins as a dichotomously branching ureteral bud originating from the posterior mesonephric duct which invades aggregates of metanephric blastema and thus initiates nephron induction. It has been established that the interaction of the ureteral bud and the metanephric blastema is a prerequisite for development of the definitive kidney (2,5,15,37,53). This interaction does not appear to require physical contact between the mesenchyme and ampullary cells but rather seems to be mediated by macromolecules produced by both structures. It has in fact been shown that nonrenal tissues are capable of inducing mesenchyme differentiation (34,51). Wessels has reported experiments in which a culture of nephrogenic mesenchyme separated from spinal cord by a porous filter, was able to differentiate into tubular structures.
These two embryologically distinct components, given the appropriate interaction, then differentiate into specific renal components. While the collecting tubules, calyces and pelvis originate from the branching ureteral bud, the glomeruli and their associated tubules evolve from the metanephric blastema.

The general pattern of nephrogenesis observed in the canine metanephros in this study agrees with that described by Potter (43), Akai (1,2) in the human and the bovine metanephros as reported by Canfield (5). However, while nephrogenesis ceases in the human metanephros beyond the thirty-fifth week of gestation (43), canine nephrogenesis continues into the second week of postnatal life (12,25).

Several studies have shown that structures such as the CNS, ovaries and the eye are especially sensitive to the effects of relatively low levels of radiation during embryogenesis (42,48). Other studies (10,11, 25,26) have demonstrated a similar radio-sensitivity during the late development of the perinatal canine metanephric kidney. In the present study we have extended these findings to include observations on radiation effects to the developing mesonephric kidney as well as to the different stages of metanephrogenesis.

The majority of animals examined 7 days after irradiation at either 21 or 28 days of age exhibited an overall reduced rate of growth and development as compared to sham-irradiated controls of similar gestation ages. In general mesonephric maturation and regression in irradiated animals appeared to lag behind that of controls by approximately 4 to 7 days. For example, 28-day-old control fetuses exhibited a well-developed mesonephric kidney with a large posterior globoid portion composed of complex, well differentiated nephrons and an anterior
portion showing marked regression; 26-day-old fetuses irradiated 7 days previously possessed a mesonephros similar to that seen in 21 to 25-day-old control fetuses. In these 28-day-old irradiated fetuses mesonephric development was limited to anterior nephrogenesis with little development of the posterior segment. Likewise, metanephric development was also delayed. In 28-day-old controls, the ureteral bud was usually well developed and had formed several distinct ampullary branches which were observed to be associated with nests of metanephric blastema accompanied by renal vesicles and occasional S-shaped nephrons. In comparable aged irradiated fetuses metanephric development was usually limited to early protrusion of the ureteral bud with little evidence of metanephrogenesis.

The most striking changes noted in animals examined 7 days after irradiation at either 28 or 35 dpc was a reduced thickness and activity of the metanephric kidney. The subcapsular layer of blastemal cells was sparsely populated and the ampullae from the ureteral bud were only partially capped by associated nephrogenic cells. In deeper zones there was a reduction in the density of renal vesicles and S-shaped structures as compared to controls.

Those findings in fetuses examined 7 days postirradiation suggest that the immediate effect of relatively low levels of radiation to the mesonephric and early metanephric kidneys is that of an interference with nephrogenesis and thus an interruption in the development and maturation of these structures. In utero irradiation, at the levels employed in this study, do not, however, appear to interfere with development of the ureteral bud and interaction with the metanephric blastema. Congenital renal agenesis resulting from the absence of the
ureteral bud has been reported in nonirradiated fetuses (44). Phemister et al. (42) in studies utilizing 150 or 250 R exposures noted uni-
lateral and occasional bilateral renal agenesis in dogs irradiated at
the time of organogenesis (21-33 days of gestation). Although they
did not elaborate further, this type of radiation-induced malformation
could result from damage to the proliferating ureteral bud, metanephric
blastema or both. Radiation-induced damage to the ureteral bud, which
occurs at approximately 21 days of gestation, should inhibit the
branching of this structure from the mesonephric duct and further
development of the metanephros.

Morphologic and morphometric studies of late gestational (55 dpc)
kidneys from animals irradiated at 15, 21, 28 and 35 days of gestation
showed no appreciable differences from sham-irradiated animals. These
observations suggest that damage to the kidneys observed 7 days post-
irradiation was reversible and did not cause permanent interference
with nephrogenesis in the developing kidneys. In previous studies
Eisenbrandt and Phemister (10,11) using dogs irradiated at 2 days of
age, observed reduced mitotic activity and extensive necrosis in the
nephrogenic zone as early as 6 hours postirradiation. Necrosis was
most evident in the tubular components of S-shaped structures, the
ampullae and renal vesicles; undifferentiated mesenchymal cells were
less affected. Nevertheless, the nephrogenic zone was conspicuous
absence by 8 days of age. In addition, dysplastic and arrested develop-
ment was noted in immature nephrons. In our study we did not observe
an obvious necrosis in the nephrogenic zone nor dysplastic corpuscles;
there was a slight decrease in the nephrogenic zone of some individual
animals irradiated at 28 and 35 days of age. The lack of visible
necrosis and cellular debris in animals examined 7 days postirradiation may be due to the fact that this interval is sufficient for removal of the necrotic debris which was still persistent in Eisenbrandt and Phemister's studies 6 hours postirradiation.

Comparison of the immediate and delayed effects of low levels of ionizing radiation to the developing kidney reveals that the effect of this irradiation may partially and temporarily interfere with nephrogenesis. Although kidneys examined 7 days postirradiation exhibited evidence of reduced renal development and arrest in nephron induction; fetuses examined in late gestation (55 day of in utero age) did not differ morphologically or morphometrically from controls. Rubin (47) found that radiation doses as low as 25 R or less may cause a mitosis-linked death of cells which primarily affects rapidly dividing cells. The cells are quickly eliminated and replaced by stimulated undamaged cells of the same type which are not injured by the radiation in a manner or to a degree that interferes with their division (47,48). This observation was supported by Eisenbrandt and Phemister (11) who observed an initial decrease in mitosis at the age of 4 days in animals irradiated at 2 days postnatally; this event was followed by an increase in mitotic activity in the nephrogenic zone that appears to decline at the age of 8 days which coincides with diminishing nephrogenesis. The present study demonstrates that stage of renal development at the time of irradiation is crucial in determining the ultimate outcome of this renal insult. It is apparent that relatively low levels of radiation at any stage of renal development result in death of mitotically active cells and an interference with nephrogenesis. As shown in previous studies of early postnatal (2dp) irradiation, this interference results
in a permanent reduction in total nephrons even though mitotic activity in the nephrogenic zone increases significantly at the age of 8 days. In this instance, however, recovery from irradiation damage occurs at the end of the normal period of nephrogenesis when other factors necessary for interaction of the ampullae and metanephric blastema have disappeared. As shown in the present study, exposure to 150 R gamma radiation at 15, 21, 28 and 35 days of gestation results in reversible damage in the nephrogenic zone of the developing kidney, however, in the case of exposure during early renal development sufficient time exists for continued nephrogenesis and the acquisition of a relatively normal metanephric kidney.


References (continued)


References (Continued)


References (Continued)


FIGURE LEGENDS
Fig. 1. Cross section of 21-25 dpc control fetus. The mesonephros is located lateral to the dorsal aorta, which supplies branches to the nephrons of the mesonephros. X95

Fig. 2. Sagittal section of the early mesonephros of 21-25 dpc control fetus. Individual nephrons are arranged in a single row. Each of the anterior glomeruli, on the right, has a single medially located mesonephric tubule. X98
Fig. 3.  Mesonephros of 21-25 dpc control fetus. Differentiated cranial nephrons are seen at the left while immature corpuscles identifiable by their poorly developed capillary networks are seen at the right. X95

Fig. 4.  The entire mesonephros of a 21-25 dpc control fetus. Well differentiated cranial nephrons are at the right, while immature nephrons are located at the upper left. X98
Fig. 5. A normal fully developed mesonephros at 26-28 dpc. In its posterior portion, the nephrons exhibit a high level of development and possess long well differentiated tubules.

Fig. 6. A normal mesonephros of the 26-28 dpc group. Early regression of the anterior region is seen where the number of nephrons is markedly reduced.
Fig. 11. A control fetus of the 32-42 dpc group. The gonad is attached to the regressing mesonephros at the left. The primitive metanephros is located at the right and in close proximity to the mesonephros and gonad. X38.

Fig. 12. Multiformed lung with branching bronchial system of 32-42 dpc control group is seen at the left. The heart is present at the center and the liver at the bottom of the field. X61.
Fig. 13. Early metanephric development of 22-25 dpc fetus is initiated by protrusion of the ureteral bud from the mesonephric duct and its interaction with the metanephric blastema. The latter appears as cellular clusters arranged around the ureteral bud. $x239$

Fig. 14. A normal early metanephros of the 25-28 dpc group. The terminal portions of the ureteral bud, the ampullae, undergo dichotomous branching while invading the metanephric blastema. An early renal vesicle is seen lateral to the ampulla on the right. $x239$
Fig. 17. A well developed kidney of an animal of the 32-57 dpc group. The outer cortex at the periphery is composed of numerous, densely packed immature nephrons, while the inner cortex possesses primarily the oldest mature nephrons recognizable by their prominent Bowman's space lined by flat parietal epithelial layer. At this stage the overall area of the cortex appears to be larger than the small mesenchymal cell medulla. 138

Fig. 18. A metanephros from a fetus approximately 42 dpc. A well delineated superficial nephrogenic layer composed of numerous immature nephron stages is seen in the outer cortex at the top. Deep to this layer are nephrons in various stages of maturation with the most mature being located closest to the corticomedullary junction. In the deep cortex corporacles are surrounded by developing tubules. The medulla at the bottom is sparsely populated by tubules which consist primarily of collecting ducts.
Fig. 21. A sagittal section of the mesonephros of a fetus irradiated at 21 dpc and sacrificed at 28 dpc. The posterior portion of the mesonephros at the left contains immature corpuscles which possess thick glomerular tufts. (Compare with the control mesonephros in Fig. 5 where the posterior mesonephros is fully developed.) X38

Fig. 22. A mesonephros of a fetus irradiated at 21 dpc and sacrificed at 28 dpc demonstrating cranio-caudal regression comparable to the control in Fig. 6. X35
Fig. 23. Posterior mesonephros at the left and early branch of the ureteral bud at right from a fetus irradiated at 21 dpc sacrificed at 28 dpc. (Compare with Figs. 15 and 16 which demonstrate the advance metanephrogenesis expressed by multiple renal vesicles and S-shaped nephrons in a control fetus of comparable gestational age.) X152

Fig. 24. Anterior regressing and posterior differentiating segments of the mesonephros with metanephros situated dorsomedially. This fetus was irradiated at 21 dpc and sacrificed at 28 dpc and represents the only animal from this group in which both kidneys resembled those of the controls. X38
Fig. 26. A metanephros from a fetus irradiated at 26 dpc and sacrificed at 42 dpc. The nephrogenic zone is thin and exhibits decreased formation of new nephrons (compare with control of comparable age in Fig. 17 where more active nephrogenic zone is observed). X152

Fig. 27. A kidney from a fetus at 28 dpc and sacrificed at 55 dpc. A slightly thinner, less active nephrogenic zone is seen in comparison to the control in Fig. 18. X61
Fig. 23. A kidney from an animal irradiated at 35 dpc and sacrificed at 55 dpc. The nephrogenic zone is less active and a large number of S-shaped nephrons are located beneath the capsule. Different immature nephron stages are seen in the inner cortex associated with poorly developed medullary rays. (Compare with the control, Fig. 18.) X61