Arsenate-induced Renal Agenesis in Rats[1,2]

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ABSTRACT The developmental origin of arsenate-induced renal agenesis was investigated. Pregnant Wistar rats were each injected once ip with 45 mg/kg sodium arsenate at day 10 (sperm day = day 0). Pregnancy was terminated at various times following injection and the embryos recovered and serially sectioned. Renal agenesis resulted when the mesonephric duct failed to give rise to a ureteric bud with subsequent failure of induction of the metanephric blastema. The underlying defect was retardation in growth of the mesonephric duct, first observed 48 hours after arsenate injection. A shortened mesonephric duct also resulted in a failure of the mesonephros to attain normal size and in the male resulted in absence of the ductus deferens, seminal vesicle and a variable portion of the epididymis. Due to the intimate association of the mesonephric and growing paramesonephric ducts, a shortened mesonephric duct resulted in a shortened paramesonephric duct with resultant lack of a uterine horn.

In recent years there has been a growing interest in studying the effects on embryonic development of a number of metals and metal-like elements found in the environment (for review of the literature see Ferm, '72, '74). Several compounds of one such element, the metalloid arsenic, are teratogenic. Sodium cacodylate induced spina bifida in chick embryos (Ancel, '46). Sodium arsenate, a compound of inorganic arsenic in the pentavalent state, has been shown to be teratogenic in the hamster (Ferm and Carpenter, '68; Ferm et al., '71) and the rat (Beaudoin, '74), while both sodium arsenate and the trivalent compound, sodium arsenite, are teratogenic in the mouse (Hood and Bishop, '72; Hood, '72).

One malformation resulting from treatment with sodium arsenate in both the hamster and the rat is renal agenesis, i.e., the absence of one or both kidneys. Renal agenesis produced as a result of teratogen administration has been reported only infrequently in the literature (Wilson, '54; Monie, '61). This study was undertaken to analyze the embryogenesis of arsenic-induced renal agenesis.

MATERIALS AND METHODS

Virgin female Wistar rats (Royalhart, New Hampton, New York) were used. The animals were maintained on Teklad Mouse and Rat Diet (Teklad Mills, Winfield, Iowa) *ad libitum* with supplemental feedings of lettuce. The day of finding sperm in the vaginal smear was designated day 0 of pregnancy. In order to establish an optimum time and dosage for the production of renal agenesis intraperitoneal injections of an aqueous solution of dibasic sodium arsenate (J. T. Baker Co., Phillipsburg, New Jersey) were given at 10:00 A.M. on one of days 9, 10, or 11 of pregnancy. Dosages of 30, 40, or 50 mg/kg maternal body weight were used. Control animals received distilled water injections or no treatment. Rats were sacrificed at day 20 at which time resorptions were counted and fetuses were examined and weighed. One-third of the living fetuses were fixed in alcohol for subsequent staining with alizarin red S for skeletal examination, and the remaining two thirds were fixed in Bouins for razor blade sectioning.

An intraperitoneal dose of 45 mg/kg of sodium arsenate was estimated to be most effective for the production of renal agenesis with a minimum of embryonic death. This dose was used to obtain specimens for the microscopic study of the genesis of absent kidney. Rats were sacrificed on one of days 11 through 16 of gestation, i.e., 24, 48, 72, etc.,

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hours after treatment. Whole embryos or posterior halves of older embryos were embedded in paraffin, serially sectioned at 7μ, and stained with stabilized iron chloride hematoxylin and PAS (Lillie, '65). Treated embryos were compared with controls of the same age.

RESULTS

The embryotoxic effect of sodium arsenate is shown in table 1. In general, numbers of resorptions increased with increased concentration of arsenate, whereas fetal weights declined under the same circumstances. Malformations produced by arsenate are given in table 2. Soft tissue malformations were seen after day 9 and 10 administration but not after day 11. Day 9 malformations involved principally the head region (exencephaly, eye defects, facial clefts) whereas day 10 defects were confined to the urogenital system. Skeletal abnormalities were common in offspring of animals treated with arsenate at days 9 and 10, but only a few minor vertebral defects were observed following day 11 administration.

Renal agenesis was produced in greatest numbers following maternal treatment at day 10. The incidence among surviving fetuses ranged from 17.3% at 30 mg/kg to 84.6% at 50 mg/kg. The incidence of bilateral agenesis increased with increased concentration of sodium arsenate. Unilateral kidney absence was not a consistent feature of either the right or the left side, and the percentage of males and females with renal agenesis was not significantly different.

Examination of day 20 fetuses with renal agenesis revealed a certain consistency in associated defects. In all fetuses missing a kidney the corresponding ureter was also lacking. Adrenal glands and gonads were present as was the bladder (even when both kidneys and ureters were absent). Ovaries and testes, however, tended to be in a higher position than normal. The incidence of nondescent of the testis was 88.9% in male fetuses with renal

TABLE 1

Embryotoxicity following maternal intraperitoneal injection of sodium arsenate

Day of treatment	Dosage (mg/kg)	No. females injected	Total implantation sites	No. resorbed or dead (%)	No. Survivors malformed (%) [1]	Fetal weight (g)
						Mean ± S.E.
	30	4	50	6 (12.0)	6 (13.6)	3.62± 0.14 [3]
9	40	8	106	56 (52.8) [2]	47 (94.0)	2.85± 0.14 [3]
	50	4	54	48 (70.4) [2]	16 (100.0)	2.98± 0.24 [3]
	30	14	195	14 (7.2)	52 (29.8)	3.66± 0.06 [3]
10	40	17	232	26 (11.2)	122 (59.2)	3.33± 0.06 [3]
	50	11	143	88 (61.5) [2]	50 (90.9)	2.96± 0.15 [3]
	30	4	49	3 (6.1)	0 (0.0)	4.00± 0.12
11	40	6	77	1 (1.3)	0 (0.0)	3.63± 0.15 [3]
	50	5	67	13 (19.4) [2]	5 (9.2) [4]	3.56± 0.06 [3]
Control		12	155	9 (5.8)	0 (0.0)	4.02± 0.06

[1] Includes both soft tissue and skeletal malformations.
[2] Percentage of resorptions significantly greater than control, $P < 0.05$.
[3] Weight significantly less than control, $P < 0.05$.
[4] Skeletal defects only.

TABLE 2

Soft tissue and skeletal malformations following arsenate treatment [1]

Day of treatment	Soft tissues					Skeleton		
	Total No. malformed	Exencephaly	Eye defects	Kidney defects [2]	Other [3]	Total No. malformed	Absent or fused ribs	Vertebral defects
9	46	18	37	12	24	23	15	22
10	133	–	–	133	–	91	91	91
11	0	–	–	–	–	5	–	5

[1] Includes all doses of arsenate. Two separate groups are recorded in this table, those examined only for soft-tissue malformations and those examined only for skeletal defects.
[2] Renal agenesis and small kidney.
[3] Agnathia, cleft lip, diaphragmatic hernia, encephalomenigocele, gastroschisis, hydrocephalus.

agenesis following 50 mg/kg of sodium arsenate (fig. 1).

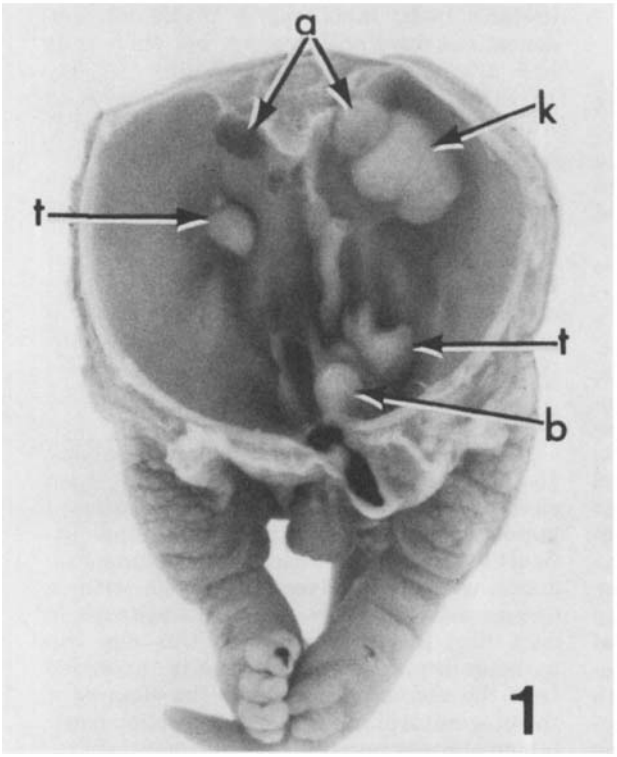
Also associated with kidney absence were defects of the tubular reproductive structures of both sexes. Day 20 male fetuses lacked the ductus deferens and seminal vesicle, as well as a varying extent of the epididymis on the affected side. Female fetuses typically had a short blind-ending oviduct close to the ovary, but in all instances the uterus was absent (fig. 2). Furthermore, when both horns of a uterus were missing, i.e., in conjunction with bilateral agenesis, the length of the vagina was reduced.

Two other urinary system defects, hypoplastic kidney and hydroureter, were seen occasionally in fetuses from rats treated at day 10. Hypoplastic kidneys were less than half the size of the control kidney, but appeared to be histologically normal. A representative female fetus with hydroureter was sectioned and it was found that the dilated ureter emptied ectopically into the vagina.

Thirty-two of fifty embryos, obtained at various times following maternal day 10 treatment with sodium arsenate, were abnormal and were used for the study of the embryogenesis of arsenate-induced urogenital malformations. The following description emphasizes specific effects of the teratogen on these embryos. Urogenital system development in control embryos was similar to that described by Torrey ('43) and Wilson and Warkany ('48).

24 hours postinjection

Six treated embryos examined at day 11 (17 to 19 somites) were not morphologically different from controls of the same age. In both the excretory system consisted of a condensation of intermediate mesoderm which extended from approximately somite 10 to the



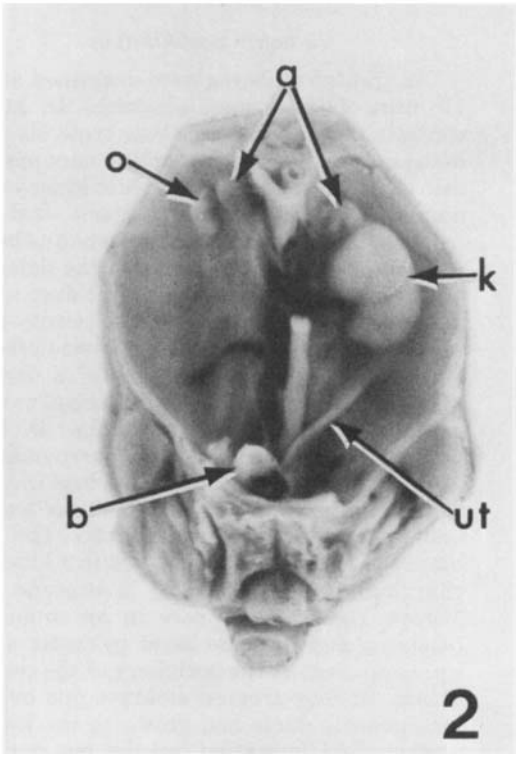


Fig. 1 Day 20 male fetus with right kidney agenesis and associated incomplete descent of the testis. The left side of the fetus is normal. k, kidney; a, adrenal gland; t, testis; b, bladder.

Fig. 2 Day 20 female fetus with right kidney agenesis and associated absence of a uterine horn. Note the high position of the ovary which is usually located behind the caudal pole of the kidney. The left side of the fetus is normal. k, kidney; a, adrenal gland; o, ovary; ut, uterus; b, bladder.

last somite. A solid cord of cells which had split from the dorsal part of this condensation was the anlage of the excretory (mesonephric) duct.

48 hours postinjection

The first abnormal embryos were seen at day 12. In controls at this stage (33 to 35 somites) the mesonephric duct extended from approximately the level of the twelfth somite to the cloaca, although fusion with the cloaca had not always occurred. The ureteric bud was recognizable in some embryos. In four of the six treated embryos there were indications of retarded growth of the mesonephric duct. In one specimen both mesonephric ducts terminated cranial to the twenty-sixth somite, which is the level of ureteric bud formation. In another embryo the right mesonephric duct made contact with the cloaca but the left one ended prematurely. In the two other specimens the right sides were comparable with controls, but the left mesonephric ducts ended in a dilation just short of the cloaca.

72 hours postinjection

Ten treated embryos were examined at day 13; nine of these were abnormal. In day 13 controls the mesonephros was typically well-developed with the mesonephric duct opening into the cloaca. The metanephric kidney rudiment, consisting of the ureteric bud and metanephric blastema, was present and beginning to differentiate. In five of the defective embryos at least one mesonephric duct terminated cranial to the level of the twenty-sixth somite and thus no ureteric bud was present. However, even in the absence of a ureteric bud, a definite metanephric blastemal condensation was always observed (fig. 3). Comparison between a blastema surrounding a ureteric bud and one without a bud revealed that an unassociated blastema was smaller and its mesenchymal cells lacked the semblance of organization observed in a blastema that had been induced by a ureteric bud. Mitotic figures were rare in an uninduced blastema and in some cases pyknotic nuclei were apparent at the periphery of the condensation. In four treated embryos one or both mesonephric ducts had grown to the level of ureteric bud formation but did not continue from this point caudalward to contact the cloaca (fig. 4). In other words, the mesonephric duct terminated as it gave rise to the ureteric bud. No differences in cell morphology, cell orientation or incidence of mitotic figures were noted between a blastema surrounding a bud derived from a mesonephric duct with cloacal contact and a blastema associated with a bud that lacked cloacal contact.

96 hours postinjection

The control mesonephros at day 14 had begun tubular degeneration and the ureteric bud had four branches within the blastema (figs. 7, 11). The paramesonephric duct first appeared as an infolding of coelomic mesothelium just lateral to the cephalic pole of the mesonephros. Of the 14 experimental embryos examined at day 14, eight were malformed. Seven embryos had shortened mesonephric ducts, failing to reach the level of kidney formation. As previously noted at day 13, a mesonephros associated with a shortened mesonephric duct was correspondingly decreased in size, i.e., no well-developed mesonephric tubules existed caudal to the termination of a mesonephric duct. In embryos lacking ureteric buds, metanephric blastemal condensations were still present, but their cells had pyknotic nuclei (figs. 9, 13). At day 14 there were two examples of embryos in which a mesonephric duct terminated as it gave rise to a ureteric bud. In one embryo the size of the associated blastemal condensation was normal but branching of the ureteric bud was reduced (figs. 8, 12). In the other embryo the ureteric bud gave rise to two very short branches that extended for less than half the distance of those in a control kidney, and a portion of the blastema of this potential hypoplastic kidney was necrotic (fig. 5, 10, 14, 15).

120 hours postinjection

Nine treated embryos were examined at day 15 and six of these were malformed. In three cases with an absent kidney the associated mesonephric duct was quite short and difficult to distinguish. These short mesonephric ducts were themselves associated with a correspondingly shortened paramesonephric duct (fig. 6). In controls at this age the paramesonephric ducts normally extended from the abdominal ostium to the vicinity of the urogenital sinus. The cranial portion had a lumen and was separate from the mesonephric duct, but the growing tip was closely applied to the mesonephric duct and the two shared a common basement membrane. In addition, in day 15 controls the ureter and mesonephric

ducts had separate openings into the urogenital sinus, but in four of the treated embryos the ureter and mesonephric duct were united close to the wall of the sinus but did not penetrate into it. The histology of the associated kidneys appeared normal.

144 hours postinjection

The day 16 control mesonephros was in the final stages of degeneration with only a few tubules remaining. The male and female gonads were easily distinguishable. The paramesonephric duct had made contact with the urogenital sinus. All five treated embryos studied had renal agenesis. One embryo was missing both kidneys and the other four were lacking one each. On the side missing a kidney it was difficult to distinguish the mesonephric duct from the few remaining cranially located mesonephric tubules, and only the ostial portion of the paramesonephric duct was observed.

DISCUSSION

In the present study the embryotoxic and teratogenic effects of sodium arsenate were similar to those reported previously for the rat (Beaudoin, '74), except that in this study numbers of resorptions were generally lower and gonadal agenesis was not observed. Sodium arsenate induced a high incidence of malformations which were dose dependent and related to the time of treatment. These malformations involved principally the head, urogenital system and axial skeleton. The incidence of renal agenesis was high enough to make the rat a suitable model for the study of the embryonic origin of this defect.

Study of embryos at successive stages of development revealed that the principal defect induced by maternal arsenate treatment was a retardation in growth of the mesonephric duct. All of the urogenital system abnormalities induced by sodium arsenate, with the possible exception of undescended testis, could be related to this condition. Renal agenesis resulted from a failure of the duct to attain the level of ureteric bud formation. When this happened no bud was formed and, consequently, no kidney was induced to develop. Nevertheless, in all arsenate-treated embryos examined at day 13 a metanephric blastema was present regardless of the condition of the mesonephric duct and ureteric bud. When a blastema was not associated with a ureteric bud, it resembled the short-lived *Zwischenblastem,* a region of nephrogenic tissue which forms neither mesonephric nor metanephric tubules (Gruenwald, '39). An uninduced blastema exhibited pyknotic nuclei at day 14 and had usually vanished entirely by day 15.

A failure of growth of the mesonephric duct has also been reported with chlorambucil-induced kidney absence (Monie, '61). Chlorambucil-induced kidney absence, however, was most often attributed to degeneration of a developing kidney whose ureter opened into a blindly-ending mesonephric duct. A similar abnormal termination of the ureter was observed in several arsenate-treated embryos, but up through gestational day 16, at least, the associated kidneys appeared histologically normal. Following onset of kidney function, however, such a situation would no doubt result in hydroureter and hydronephrosis.

It has been suggested that kidney hypoplasia results from a failure of induction of the entire metanephric blastema by the ureteric bud (Crocker et al., '71). In our study one treated embryo examined at day 14 appeared to exhibit a stage in the development of a hypoplastic kidney. In this embryo the portion of the metanephric blastema most distal to the ureteric bud was necrotic and resembled an uninduced blastema, a finding which suggests that the inductive effect of the bud was not manifested in this area. Brown ('31), in her study of a mouse mutant with kidneys of various sizes, proposed that abnormal penetration of the blastema by the ureteric bud may occur when the bud is shortened or delayed in reaching the blastema. In light of the inductive interdependence known to exist between the two elements which make up the developing kidney (Grobstein, '55), a shortened or delayed ureteric bud could result in an incomplete induction of the blastema which would, in turn, result in the induction of fewer branches of the ureteric bud and, consequently, the appearance of fewer metanephric tubules.

Another consequence of retarded mesonephric duct growth was a reduction in the number of mesonephric tubules. It has been demonstrated in amphibian (Cambar, '48) and chick (Calame, '59) embryos that when a growing mesonephric duct is surgically obstructed, a correspondingly reduced mesonephros will result. This has been interpreted as evidence that the mesonephric duct induces mesonephric tubule formation in the same way that the

ureteric bud induces metanephric tubule formation. Since the cranial end of the mesonephros is induced first by the growing mesonephric duct, the efferent ductules of the testis, which are derived from these most cranial mesonephric tubules, and a shortened portion of the duct of the epididymis were normally present in day 20 males with renal agenesis. However, the ductus deferens and the seminal vesicle, which arises as a bud from the ductus, were consistently absent.

It is known from the work of Gruenwald ('37, '41) that, except for the abdominal ostium, the paramesonephric duct is dependent on the mesonephric duct for normal development. The growing tip of the paramesonephric duct is found within the basement membrane of the mesonephric duct and, for this reason, is unable to continue its growth in the absence of the duct. Consequently, in arsenate-treated embryos the paramesonephric duct was affected to the same extent as the mesonephric duct, and, therefore, kidney and uterine horn were both absent on the affected side. The short segment of oviduct seen in day 20 female fetuses with renal agenesis corresponds to the ostial portion of the paramesonephric duct which forms independently of the mesonephric duct. The intimate relationship between mesonephric and paramesonephric ducts also provides a possible explanation of how a ureter could open ectopically into the vagina (which is derived in part from the paramesonephric ducts). This condition was observed in one serially-sectioned fetus with hydroureter. Normally, by the time a paramesonephric duct has reached the urogenital sinus the ureter and the mesonephric duct already have separate openings into the sinus. However, in the commonly observed situation where the mesonephric duct gives rise to a ureteric bud but fails to contact the urogenital sinus, the close contact between the mesonephric and the paramesonephric ducts could result in a ureter emptying into the vagina by way of a short extent of mesonephric duct. In the fetus studied a change in epithelium from transitional to simple columnar indicated the transition from ureter to mesonephric duct.

An anatomical explanation for the incomplete descent of the testes, often associated with renal agenesis, was not evident from microscopic observation of developing embryos. Normally, the rat testes descend between days 16 and 19 and by day 20 are located in the inguinal region (Wilson and Warkany, '48). Since differential growth is the critical factor in testis descent, a general growth retardation in the caudal region of the fetus may interfere with the normal descent process. Reduced numbers of somites observed in arsenate-treated embryos and low fetal weights suggest that a general growth retardation occurred.

Sodium arsenate-induced renal agenesis in the rat does not duplicate exactly the condition seen in humans with congenital kidney absence. It is estimated that in humans unilateral kidney absence occurs once in every 1,000 births with the defect more common in males and the left kidney missing more often (Vaughan and Middleton, '75). In contrast, after arsenate treatment there was no significant difference between the incidence of renal agenesis in male and female offspring and neither side was consistently affected. In humans bilateral kidney agenesis is associated with a syndrome of defects (Potter, '72). These defects, including pulmonary hypoplasia, bowed legs and a typical facies, have been attributed to a characteristic scarcity of amniotic fluid associated with this condition (Fantel and Shepard, '75). Amniotic fluid volume in hamsters with arsenate-induced bilateral agenesis was not significantly different from controls (Ferm and Saxon, '71) and, likewise, in the present study there was no apparent reduction in volume of amniotic fluid observed at the time of recovery of fetuses.

Several human embryos which depict stages in the development of renal agenesis similar to those seen in the rat after arsenate administration have been described (Boyden, '32; Gruenwald, '39; Aüer, '47). In all three embryos defective mesonephric duct or ureteric bud development was observed. In addition, renal agenesis accompanied by other urogenital abnormalities, including absence of a ureter, uterine horn and ductus deferens occurs spontaneously in one rat strain (Fujikura, '70; Cramer and Gill, '75).

The teratogenic mechanism of action of arsenic is not known. In general, the actions of all arsenicals have been attributed to the trivalent forms which are thought to exert their effects by reacting with sulfhydryl groups in cells (Harvey, '75). The pentavalent arsenate and the trivalent arsenite are interconverted in vivo. Arsenite inhibits the oxidation of pyruvate to acetyl Co A, a reaction which is an

obligatory step for entry of carbohydrates into the Krebs cycle (Webb, '66). Interference with this cycle could result in a reduction of energy available to the embryo. It is of interest that sodium arsenite administered to day 10 pregnant rats, although highly toxic, did induce a low incidence of renal agenesis in surviving fetuses (Burk, unpublished).

The specificity of arsenic for the developing kidney also demands further investigation. Renal agenesis and related malformations observed after sodium arsenate treatment resulted from a defective mesonephric duct which was unable to grow to the level of ureteric bud formation. A general energy reduction which results in decreased cell proliferation could be postulated to explain this defect, but the characteristic site-specificity of arsenate teratogenicity seems to suggest the involvement of a more specific mechanism.

Furthermore, preliminary information derived from the use of a radioisotope of arsenic (^{74}As) indicates that the rat placenta is permeable to arsenic, but only a very small quantity is present in the embryo at the time when renal agenesis is induced (Burk, unpublished). Whether this quantity of arsenic is sufficient to be responsible for the malformation has not yet been determined, and it is still possible that the effect of arsenic on the embryo may be due to an interference with maternal metabolism or placental function.

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PLATE 1

EXPLANATION OF FIGURES

Figs. 3-6 Schematic diagrams of some defects observed in embryos at various times following maternal treatment with sodium arsenate at day 10. In each figure the left side depicts the control urogenital system at the day indicated. The mesonephric ducts and ureteric buds are white, the metanephric blastemata are stippled, the mesonephric tubules (mesonephros) are striped, the urogenital sinus is labeled s, and the paramesonephric ducts are black.

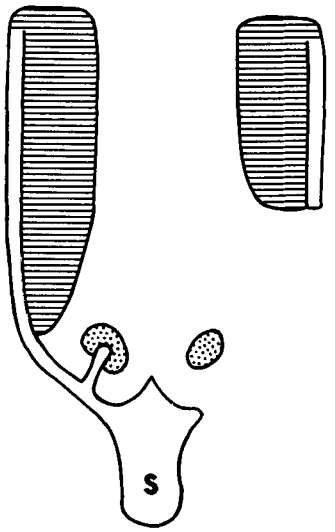
3 Failure of the mesonephric duct to attain the level of ureteric bud formation. There is no ureteric bud, but the metanephric blastema is still present. The length of the mesonephros reflects the premature termination of the mesonephric duct.

4 The mesonephric duct attains the level of ureteric bud formation but fails to make cloacal contact. The kidney appears to begin a normal development. The length of the mesonephros is not affected.

5 This potential hypoplastic kidney is only half the size of a control kidney. In the embryo on which this diagram is based the mesonephric duct failed to make contact with the urogenital sinus.

6 The mesonephric duct is quite short and no metanephric blastemal cells remain. The paramesonephric duct reflects the defect in the mesonephric duct. Only the ostial portion is present.

Day 13



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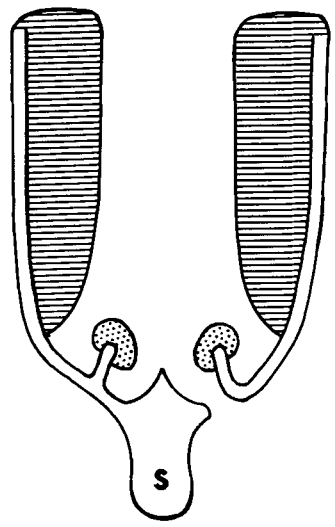
Day 13



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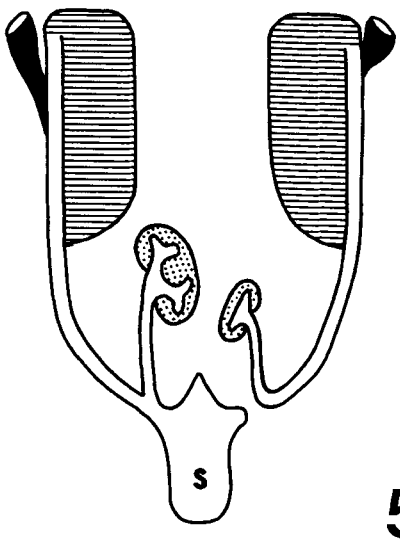
Day 14



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Day 15

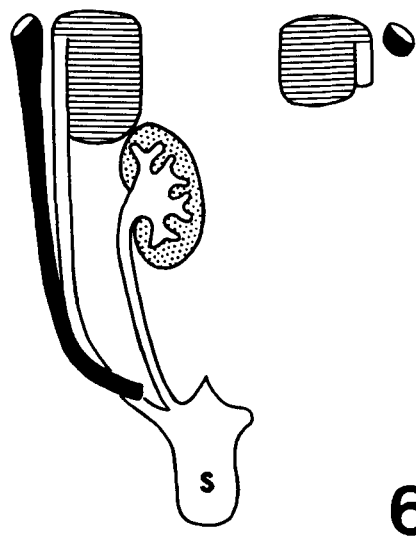

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PLATE 2

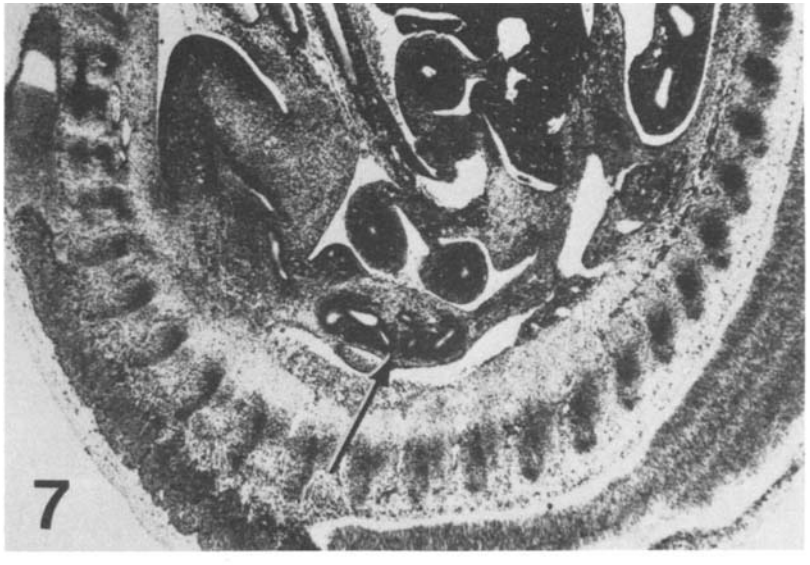
EXPLANATION OF FIGURES

7 Sagittal section through a day 14 control embryo. Note the size and branching pattern of the developing kidney at the arrow. × 40.

8 Sagittal section through an arsenate-treated embryo examined at day 14. Although the extent of this developing kidney (indicated by the arrow) is comparable with that of the control, the number of branches of the ureteric bud is reduced. × 40.

9 Sagittal section through an arsenate-treated embryo examined at day 14. In this embryo a mesonephric duct failed to reach the level of ureteric bud formation. A degenerating metanephric blastema is present at the arrow. × 40.

10 Sagittal section through an arsenate-treated embryo examined at day 14. A potential hypoplastic kidney is located at the arrow. This section shows the full extent of the developing kidney. × 40.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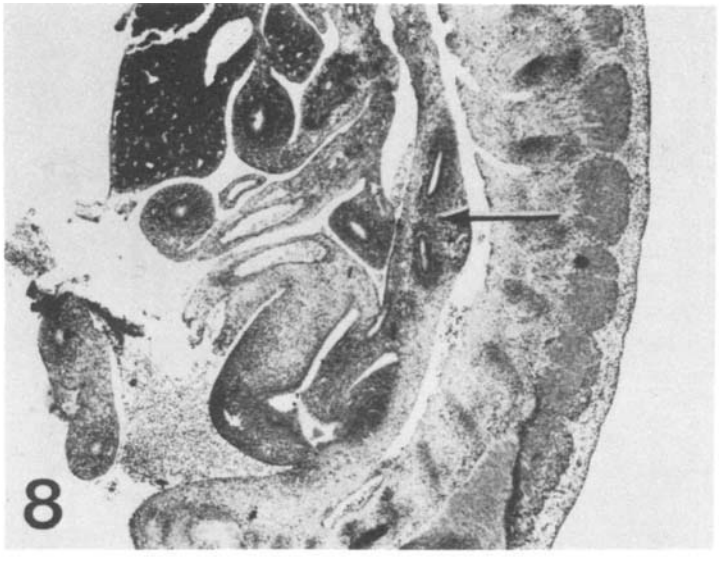
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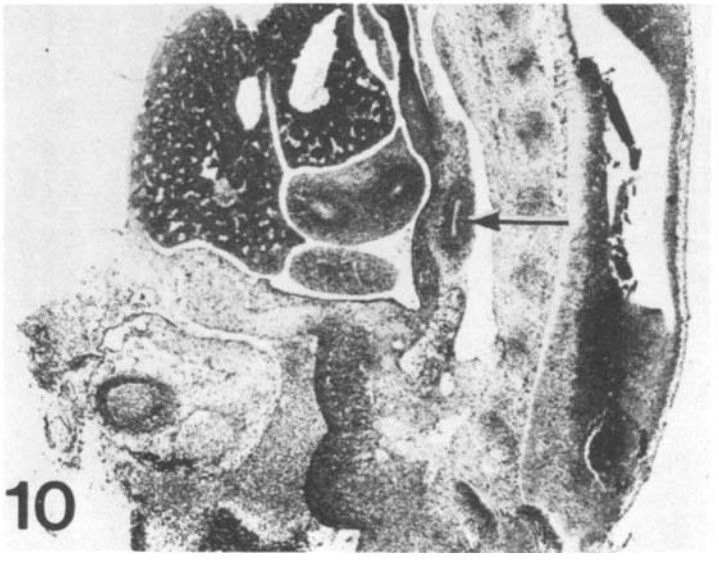
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PLATE 3

EXPLANATION OF FIGURES

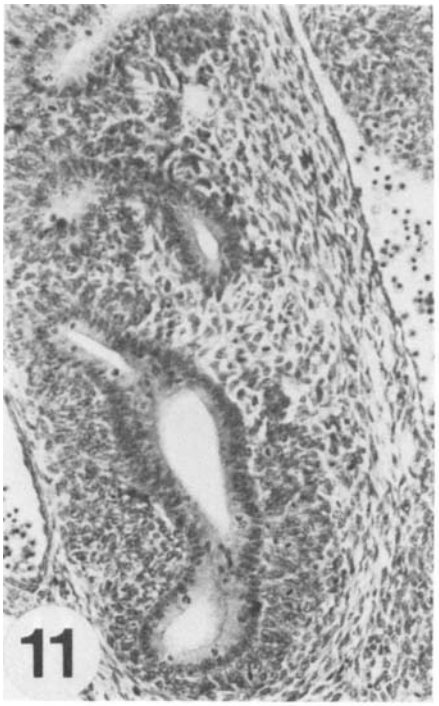
11 Higher magnification of 14-day control kidney seen in figure 7. Note mitotic figures along the lumens of ureteric bud branches and in the surrounding condensed mesenchyme. × 200.

12 Developing kidney (day 14) from the arsenate-treated embryo seen in figure 8. Although the kidney is less complex than the control (fig. 11), the cells appear healthy. × 200.

13 Higher magnification of the degenerating metanephric blastema (day 14) seen in figure 9. Note numerous pyknotic nuclei visible in the condensed mesenchyme of the blastema. × 200.

14 Higher magnification of the potential hypoplastic kidney (day 14) seen in figure 10. At this point the kidney, although quite small, appears healthy. (Compare to figure 15). × 200.

15 Section from the same kidney seen in figure 14. This portion, which is farther distal to the ureteric bud, exhibits numerous pyknotic nuclei and resembles the degenerating metanephric blastema seen in figure 13. × 200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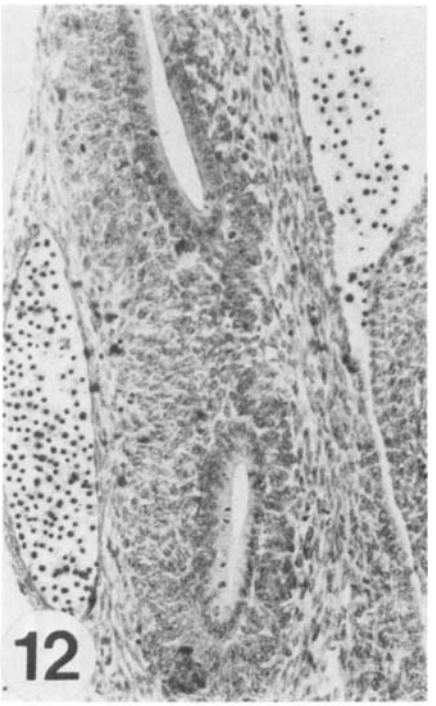
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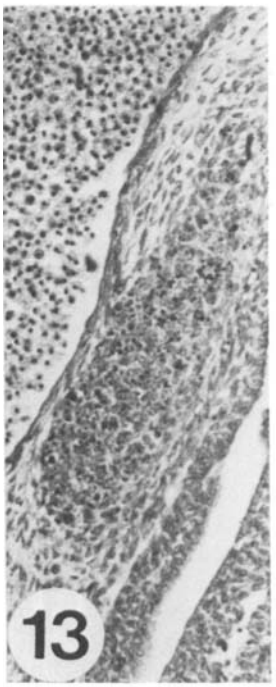
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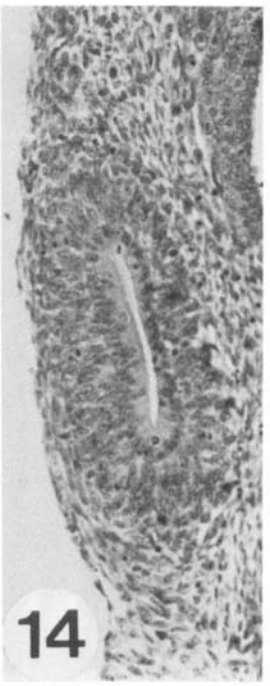
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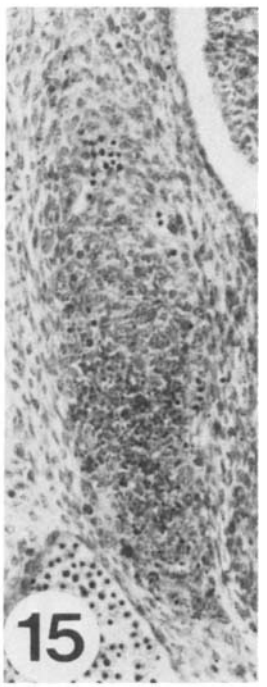
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