

THE PRODUCTION OF  
INTRAUTERINE URÉTERAL OBSTRUCTION AND VESICO-URETERIC  
REFLUX AND THEIR EFFECT ON THE FOETAL KIDNEY

**CENTRE FOR NEWFOUNDLAND STUDIES**

**TOTAL OF 10 PAGES ONLY  
MAY BE XEROXED**

**(Without Author's Permission)**

SUNMOLU AKINLOLU BECKLEY



385587







THE PRODUCTION OF  
INTRAUTERINE URETERAL OBSTRUCTION AND VESICO-URETERIC REFLUX  
AND THEIR EFFECT ON THE FOETAL KIDNEY

by

SUNMOLU AKINLOLU BECKLEY, L.R.C.P.S.I.

A Thesis

submitted in partial fulfillment of the requirements

for the degree of

Master of Science

in

The Faculty of Medicine

Memorial University of Newfoundland

St. John's, Newfoundland

July, 1974



## TABLE OF CONTENTS

ABSTRACT	i
ACKNOWLEDGMENTS	iii
LIST OF FIGURES	v
CHAPTER 1	1
ANATOMY	4
Development of Kidney in Man	4
CHAPTER 2	13
MATERIAL	13
Supply of Model	13
Choice of Model	13
Breeding	14
METHOD	17
Preparation for Surgery	17
Anaesthesia	18
SURGERY	21
Group A - Ureteral Ligation	21
Closure	23
Group B - Creation of Vesico-ureteric reflux	24
Closure	31
Post-Operative Care	32
RESULTS AND DISCUSSION	33
URETERAL OBSTRUCTION	34
MICROSCOPIC	34
CONCLUSION	44
REFERENCES	46

## ABSTRACT

The unborn fetuses of sheep have been used as an experimental model in the study of certain congenital renal anomalies. Monoestrous ewes were bred on a farm and radiographed at about 60 days gestation for confirmation of pregnancy. Using epidural anaesthesia, each ewe was submitted to hysterotomy at about 90 days gestation to perform one of two types of studies on the foetus.

The first experiment, that of ureteral obstruction, involved delivery of the caudal half of the foetus into the wound and approaching the left ureter from the back, lateral to the lumbar vertebrae. A silk ligature was placed on the ureter and the foetus and maternal wounds were closed separately.

The second experiment was one of creating vesico-ureteral reflux in the unborn foetus. The foetus was delivered and a lower vertical midline, or in the male paramedian, incision was made anteriorly and the bladder entered. The ureteral orifice was unroofed adequately to leave a gaping hole. The bladder and foetal wound were closed separately and the closing procedure was carried on as in the first experiment.

The experience gained in these studies suggested that preliminary radiography provided both an excellent confirmation of pregnancy in the ewe and also the number of fetuses present. Adequate premedication with Chlorpromazine and the use of epidural anaesthesia avoided inhalation of vomitus, jeopardizing the foetus with drugs that cross the placental barrier, and provide good



anaesthesia. Amniotic fluid loss could be circumvented by using the method adopted here. Because of the friable gelatinous nature of the foetal tissue, surgery is not practicable before 70 days gestation. Whereas hydronephrosis resulted from ureteral ligation in these cases, earlier ureteral ligation may result differently. Creation of vesico-ureteral reflux was accomplished in utero and the effects of this are yet to be evaluated.



## ACKNOWLEDGEMENTS

This work would not have been started, yet alone completed without the untiring help so freely given by a number of people.

Firstly, my thanks go to the Medical Research Council of Canada for providing me with a research fellowship for the duration of this project. Next, the Departments of Surgery and Radiology at Memorial University of Newfoundland for providing funds for equipment, materials and travel as well as for laboratory space and the use of their facilities.

My wholehearted gratitude and indebtedness go to my Supervisors who got this work off the ground and were closely involved with every phase of it, making themselves available to give advice and sincere help that I needed at all times. They were extremely patient and resourceful all the way.

Dr. Patrick McManamon was of great assistance technically at surgery and also in his field of radiology. The technicians in the research laboratories, Mrs. Jean Banks, Miss Hilary Lewis, and Mrs. Jan Williamson gave of their time and technical expertise beyond the call of duty in assisting with surgery and radiography, as well as looking after the experimental models before and after surgery.

Mrs. Olive Churchill was very patient and helpful with the secretarial work involved, as was Miss Judy Cresanti who did the final stages of the work. My thanks go to Funlola, my wife, for being so patient and understanding.

Last, but not least, are a number of people who voluntarily gave their help, amongst whom are Dr. Martin Lewis and his staff, Dr. Yves LeGal, Mrs. Gallagher and her staff. No doubt I have omitted the names of some people who gave substantial help for which I am indebted but space would not permit me to mention them all.

## LIST OF FIGURES

Figure 1	Origin and Early Development of the Kidney	6
Figure 2	A. Origin of Collecting Ducts B. Origin of Bowman's Capsule C. Origin of Tubule	8
Figure 3	A. Advancement of Nephrons B. Arcade Formation of Nephrons C. Kidney: Arrangement of Nephrons at Birth	9
Figure 4	Radiograph of Pregnant Ewe	16
Figure 5	Placement of epidural space needle and infection of 10 cc. xylocaine with 1:200,000 epinephrine into the space	20
Figure 6	Delivery of Uterus into Operating Field	25
Figure 7	Delivery of Foetus into Operating Field	26
Figure 8	Delivery of Caudal Half of Foetus into Operating Field	27
Figure 9	Exposure of Foetal Urinary Bladder	28
Figure 10	Closure of Foetal Wound Using Continuous Interlocking Sutures	29
Figure 11	Closure of Uterine Wound	30
Figure 12	Intravenous Pyelogram (40 minute film) of a 24 hour Old Lamb with Left Ureteral Ligature	35
Figure 13	Right Vesico-Ureteral Reflux - Intravenous Pyelogram within Normal Limits on First Day of Life	36
Figure 14	Right Vesico-Ureteral Reflux: Micturating Cystogram	37
Figure 15	Left Ureteral Ligation: Fresh Autopsy Specimen	38

(Continued)



Figure 16	Left Ureteral Ligation	39
Figure 17	Left Ureteral Ligation	40
Figure 18	Left Ureteral Ligation	41
Figure 19	Left Vesico-Ureteral Reflux with Minimal Increase in Size of Kidney	43



In the search for a plausible explanation for the aetiology and pathogenesis of various congenital abnormalities of the urinary tract in man, investigators have directed their attention to the scrutiny of the prenatal period. Having established the normal embryological processes, various anomalies have been simulated by interrupting particular processes in the development of the foetus. Various animals have been used as experimental models but the more convenient ones have been the dog, rabbit and sheep.

Foetuses were first subjected to surgical experimentation by Mayer, A. (1915) who castrated a foetal dog and placed the foetus in the maternal peritoneal cavity to continue its development. Peritonitis ensued, causing maternal death two days after surgery. Wolff, B. (1919) decapitated a rabbit foetus in utero with survival for 8 1/2 hours. This was the first reported wholly intrauterine experiment. Several investigators then became interested in this new experimental approach. Nicholas, J.S. (1925) amputated the forelimb of 104 foetal rats 1-9 days prior to delivery with a 58% survival rate. He suggested that the nearer to term the surgical intervention, the greater the chances of foetal death. In 1934, he presented a second series of 104 foetal rats in which surgery was performed between the 14th-20th days of gestation with a 70% survival rate. Bors (1925) subjected foetal rabbits to surgery in utero. He amputated a limb and used a special device to keep the foetus within the uterus during surgery. Hooker, D. and Nicholas, J.S. (1930) reported 293 foetuses which had their spinal

cords sectioned in utero between 12-18 days gestation. Mortality was low, at 15%. Tobin, C.E. (1939) attempted to destroy the adrenals of a rat foetus in utero, using cautery, at 17 days gestation. 40% of 402 foetuses survived, but in only 3 (1.9%) were the adrenals destroyed. Jost, A. (1946 and 1947) reported submitting foetal rabbits to parabiosis, and others (117) to gonadectomy between the 19th-24th day of gestation. He also gave a group of the gonadectomized rabbit foetuses a pellet of androgen, inserted subcutaneously. All his experiments were terminated between the 26th-28th day of gestation. He had a 56% survival rate. Of note is the fact that only the caudal end of the foetus was allowed to emerge from the uterus at any time during the surgery.

Wells, L.J. (1950) reported the work he did from about 1945, castrating rat foetuses and leaving them in the maternal peritoneum to grow. Barnard, C.N. (1955, 1957) reported the work he did, first in South Africa and then at Minnesota, in an attempt to clarify the aetiology of intestinal atresia. He ligated one or more ileal vessels where their branches arise to supply the small intestine. He was able to demonstrate the feasibility of the experiment and also showed that amniotic fluid loss can be minimized by gentle upward traction on the maternal uterus. Hitherto, excessive loss of amniotic fluid had been associated with a high mortality rate of the foetus. The mechanism of this is not completely understood but sudden changes in hydrostatic pressure, heat and fluid loss, as well as greater



exposure to transmitted pressure changes may be responsible factors.

Barcroft, J. and Barron, D.H. (1936) described operations on foetal lambs using spinal anaesthesia on the mother.

Cowen, R.H. and Laurensen, R.D. (1959) exposed the entire rabbit foetus in a study of the aetiology of congenital muscular toticollis. Jackson, B.T. and Egdahl, R.H. (1960) marsupialized the foetal skin to the uterine wall in an attempt to reduce the loss of amniotic fluid to a minimum. The necessity to keep amniotic fluid loss to a minimum was denied by Jost, A. (1946), Wells, L.J. (1950), Cowen, R.H. et al (1959), Blanc, W.A. and Silver, L.A. (1962), Morgan, W.W., Rosenkrantz, J.G. and Hill, R.B. Jr. (1966), Rosenkrantz, J.G., Simon, R.C., Carlisle, J.H. (1968).

Beck, D.A. (1970, 1971) suggested aspirating the amniotic fluid and placing it in a sterile dish containing antibiotics, thus bringing the sterile technique up to that expected in human surgery. He also avoided using general anaesthesia. Unlike the work of Barcroft, J., Barron, D.H. and Windle, W.F., working in Cambridge in 1936, who used "1.5 - 3 cc of 'durocaine' intraspinally in the lumbar region", Beck carried out epidural anaesthesia, using lignocaine. Thomasson, B.H., Esterly, J.R. and Ravitch, M.M. (1970) reported their work on rabbits using general anaesthetic in their study of renal morphological changes secondary to ureteral obstruction.

It appears therefore that to circumvent the problems encountered by previous workers attention to certain details is essential. These

include: an experimental model that is easily obtainable, of practical size, and with predictable anatomical and physiological properties; detailed attention to anaesthesia, particularly the avoidance of agents which may pass through the placental barrier with injurious effect on the foetus; and prevention of regurgitation and aspiration of stomach contents. Surgical technique must prevent loss of amniotic fluid, foetal or placental injury and at the same time accomplish the necessary procedures. Asepsis as well as standard post-operative care are also essential for a successful outcome.

The object of the present study was to obtain definitive data on the morphological changes resulting from ureteral obstruction in the unborn foetus, with the hope of demonstrating some aspect of the pathogenesis of one of the forms of cystic disease that occur in the human infant.

## ANATOMY

### Development of Kidney in Man

The mesoderm of the nephrotome gives origin to urine-producing structures which later form the pronephros, the mesonephros, and finally the metanephros. The pronephros first appears early in embryonic life, developing cephalad and independently. It is made up of 6 to 10 pairs of tubules with a connecting duct called the excretory, or Wolffian, duct which opens into the coelomic cavity.

The pronephros degenerates by about the fourth embryonic week leaving only the excretory duct to develop further by extending



caudally through the nephrogenic cord to open into the cloaca.

The mesonephros, which is longer and more caudally situated than the pronephros, makes its appearance just before the pronephros disappears. It originates from the mesoblastic intermediate cell mass and mostly consists of tubules at the end of the mesonephric duct (Wolffian). Early on it appears opposite the upper thoracic segments but elongates to the level of the third lumbar vertebra (Fig. 1). At this stage, glomeruli are present internally, along with collecting tubules which drain urine into the cloaca via the mesonephric ducts. Degeneration of the mesonephros occurs by about the 14th week of gestation and is maximal by the 16th week leaving a residuum of small collecting tubules. These remnants persist as the epoophoron in the female and as the ductuli efferentes, ductuli aberrantes and paradidymis of the epididymis in the male.

The metanephros is the true renal secretory anlage, consisting of a glandular or secretory portion and an excretory portion. The excretory portion, i.e. ureter, pelvis, calyces, papillary ducts and straight collecting tubules arises from the ureteric bud, whereas the glandular portion, or nephron, arises from the caudal end of the nephrogenic cord.

The metanephros appears caudally at the level of the 2nd sacral segment during mesonephric degeneration as the renal blastema (nephrogenic cord). The ureteric bud emerges from the mesonephric duct and soon becomes surrounded by, and fuses to, the elements of the

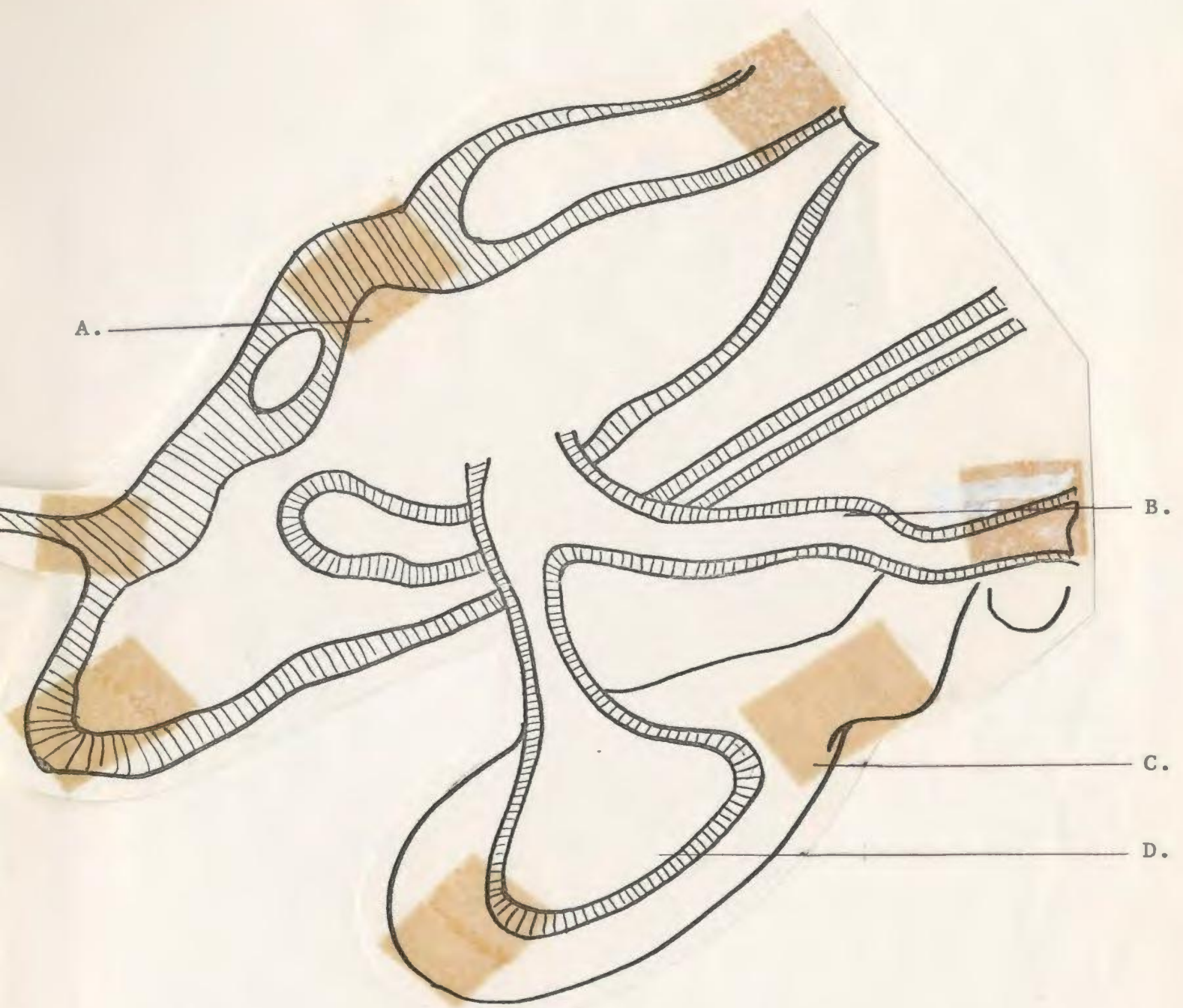


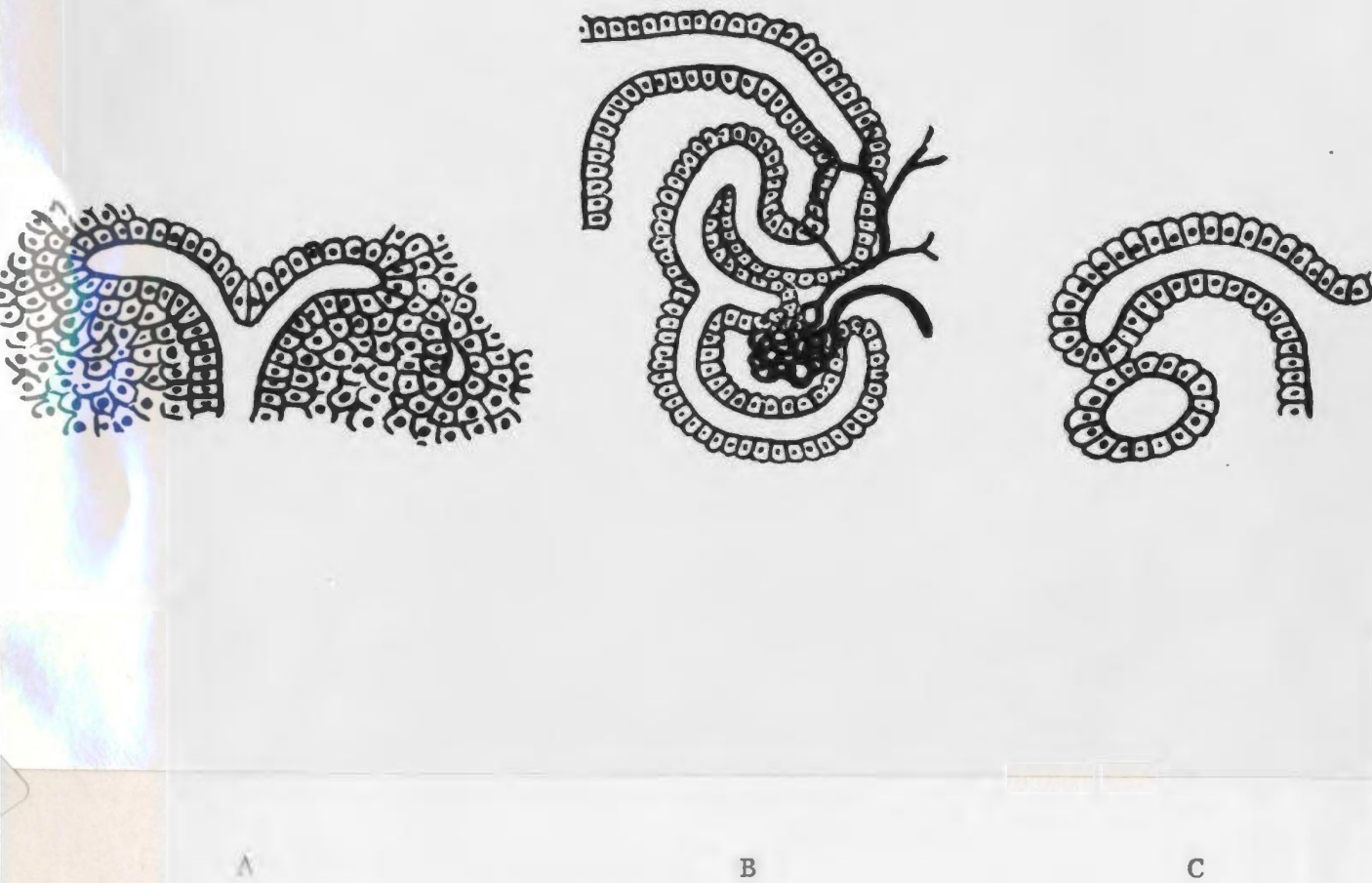
Figure 1 Origin and Early Development of the Kidney

- |                         |                     |
|-------------------------|---------------------|
| A. Cloaca               | B. Nephrogenic Cord |
| C. Metanephrogenic Mass | D. Ureteric Bud     |



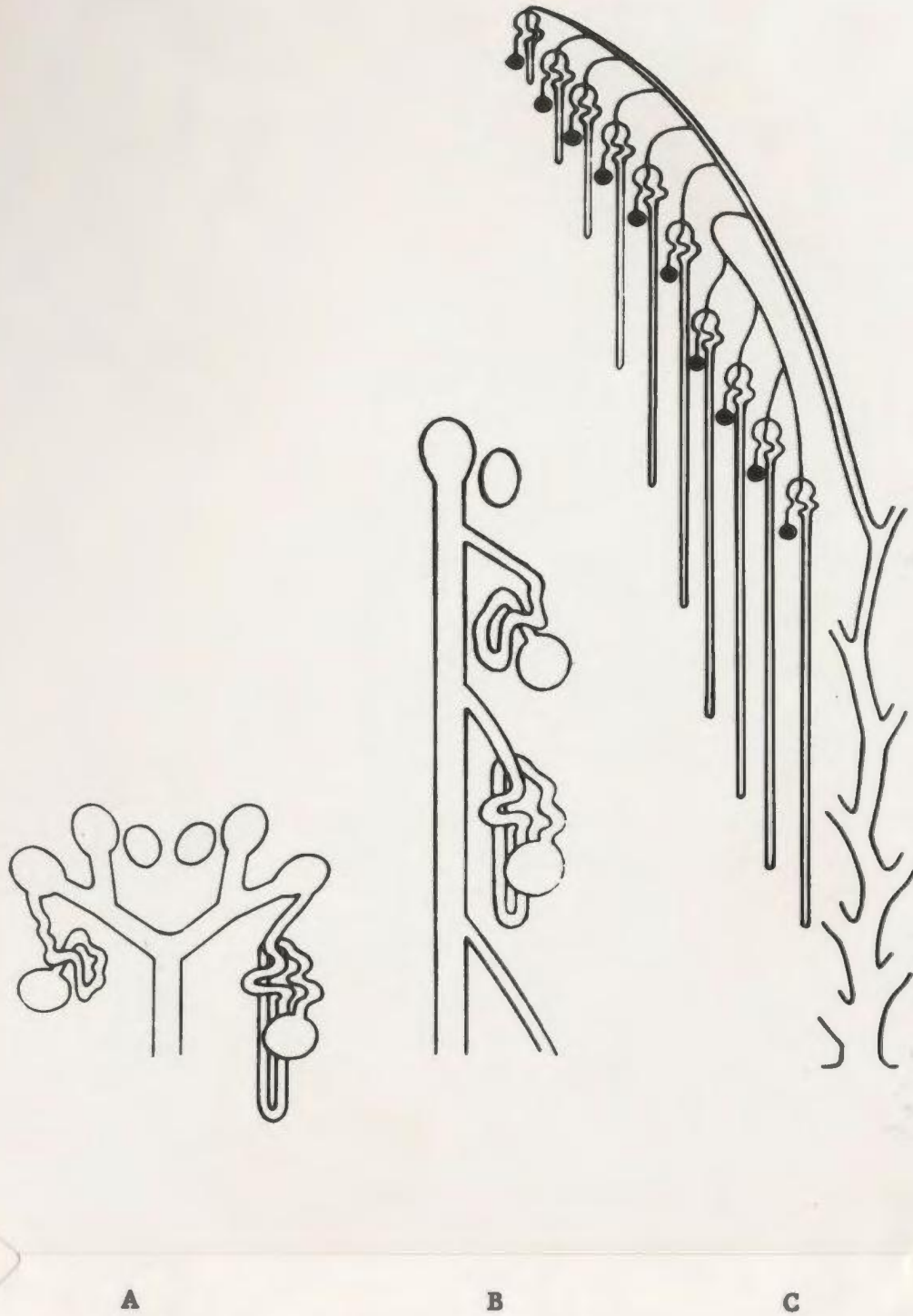
caudal portion of mesonephric cord, thus establishing the drainage system. At the same time, vascularization develops in such a way that by the time the nephrons are established urine filtration occurs. By division and subdivision of the renal end of the ureteric bud the major and minor calyces are formed as well as the expanded portion, designated the pelvis (Fig. 2A). By about the 7th week of gestation, further divisions occur giving rise to the primary collecting tubules (Figs. 2B & C). These, in turn, divide to form the secondary and tertiary tubules and further branches until, by the 5th month, upwards of 12 divisions are established. The primary collecting tubules form the major calyces, the secondary collecting tubules the minor calyces, and these later absorb the third and fourth orders of tubules, thus directly receiving the fifth order. By this means, the fifth order of tubules form the papillary ducts. Higher orders of tubules form the straight collecting ducts which, along with the loops of Henle, form the medulla. These and their branches project into the cortex to form the para radiatia, or the medullary rays (Fig. 3). The tubular complex thus formed and draining into a minor calyx constitutes a pyramid whose apex, or papilla, projects into the calyx while the base is surrounded by the cortex. Subsequently each pyramid, without its papilla, divides into several secondary pyramids draining into a single papilla.

Of interest is the work of Osathanondh, V. and Potter, E.L. (1963) who studied, by microdissection, kidneys ranging from an 11 mm



**Figure 2** A. Origin of Collecting Ducts - Collecting Tubule is Surrounded by Metanephrogenic Tissue  
B. Origin of Bowman's Capsule  
C. Origin of Tubule





**Figure 3**    **A. Advancement of Nephrons**  
              **B. Arcade Formation of Nephrons**  
              **C. Kidney: Arrangement of Nephrons at Birth**

embryo to those of a 78 year old man. These studies suggested that the ureteral bud divided into two branches upon entering the metonephric blastema at 6 weeks gestation. These two branches further divide, the polar ends more rapidly dividing but shorter in length than the mid portion. By the 10th to 14th weeks the renal pelvis becomes distinguishable. Tubules of the third and fourth generations (branchings) become dilated, the proximal ends opening into the pelvis in the mid portion, and the distal ends becoming the minor calyces which number about two to twenty-five. At the polar ends of the renal mass the third, fourth, and fifth generations enter into the formation of the renal pelvis, although the fifth generation forms the major calyces, which number about four to ten.

Upon formation of the major calyces three to five more divisions occur throughout the kidney. Thus many tubules emerge from a common stem and, since nephrons and tubules have been formed, urine excretion may result in tubular dilatation. Fifteen to twenty nephrons are formed simultaneously and drain into a single tubule.

From the 14th to 22nd week of gestation new nephrons are formed and are arranged in arcades, draining into a common stem separate from the main collecting duct. The most recently formed are joined to the ampullae or growing ends of the branches of the ureteric bud. At the 22nd to 36th week fresh ampullae are formed beyond the point at which the arcades are attached, and drain directly into the common

collecting duct. The terminal unbranched parts of the tubules are formed about this time and the glomeruli of these lie in the outer part of the cortex. These form the final phase of nephron formation and the ampullae disappear at this stage. Each collecting tubule now represents the 6th to 9th generation of branches distal to the minor calyx and has 10 - 14 nephrons (i.e., 4 - 7 on each side arranged in an arcade). Differentiation of renal tubules stops at the 8th month and at this time each kidney has a million nephrons.

Thus Osathanondh and Potter (1963) divided the embryogenesis of nephrons into 4 periods. The first period starting at the 5th week of gestation and lasting till the 14th to 15th week. Here the growing end of the ureteric bud, known as the ampulla, divides frequently inducing formation of nephrons which remain attached permanently to the ampulla of origin. If these nephrons do not advance they degenerate. In period 2, lasting from 14th to the 22nd week of gestation, ampullae seldom branch. New nephrons are induced repeatedly by ampullae already carrying attached nephrons. The collecting parts of the nephrons communicate with each other to form arcades.

In period 3, lasting from the 22nd week to the 36th week, the ampullae almost never branch. They advance beyond the point of the arcades and induce formation of nephrons which become attached to the ampullae. These nephrons are left behind as the ampullae advance thus producing several generations attached in orderly succession to the



terminal portion of each collecting tubule. Ampullae disappear at the end of period 3. In period 4 starting from the 36th week till adulthood, kidney growth is due to enlargement of tubules already present.

It is therefore feasible to count the number of nephrons and thus determine the stage of growth of the kidney. It would also be feasible that cutting the kidney was suggested by Hodson, C.J. (1972) transversely and parallel to the renal vessels that one can see the glomeruli arranged in an arcade fashion. Thus, if there is cortical atrophy, this would be amply evident by a reduction in the number of nephrons.

## CHAPTER 2

### MATERIAL

#### Supply of the Model

Sheep were obtained locally from farmers, the criteria being that they should either have had a previous successful pregnancy, or never been bred but were known to come from a good flock. Enquiries were also made to ensure that one did not obtain known aborters. Here we came up against a lot of problems, because this region of Canada is not a sheep-rearing area, and to keep costs down to a minimum, it was decided to get the best we could from the limited local supply. It became evident, late in the experiment, that several of the flock came, after all, from a farm in which there had been several abortions the previous year.

#### Choice of the Model

The choice of sheep over other animals as our experimental model was based on the fact that young primiparous sheep usually have only one lamb, and although twinning is rampant in second and subsequent pregnancies, one would rarely ever have the problems of the multiple foetuses that occur in pigs, dogs and rabbits. The sheep foetus is usually of good size and should present no problems in surgery. The gestation (147-150 days) is a little long but it allows of some leeway in planning the surgical intervention. The only other problem is that the sheep, being a ruminant, is more likely to regurgitate the stomach contents, and there is always a fear of intrapulmonary aspiration in these cases.



### Breeding

Ten ewes were placed in a small barn with a ram which had its ventral aspect painted with red oak (a red marking-paint used by the local fishermen in marking their fishing nets). We had to resort to this because the ewe marker that had been ordered was not available at the start of the breeding season. The red oak and the ewe marker work on the same principle, except that the red oak had to be replenished daily. Each time a ewe was mounted by the ram, the paint on the ram left its mark on the dorsocaudal aspect of the ewe. This was taken as a presumptive evidence of insemination. It is a common practice in animal husbandry to accept this presumptive evidence. The ram is known to ignore the ewe if she is either pregnant or is not in the oestrus cycle. This cycle lasts 14-19 days and usually occurs early in frosty weather, which is between September and January in this part of Canada. (It is possible to obtain a breed of sheep (dorset horn) known to be polyestrous and can be brought into heat twice yearly.)

The flock with the ram was examined each evening and morning and any evidence of breeding noted. If this was convincing, the ewe that was bred was removed and a tag was placed around its neck. The tag number and date of breeding were recorded. If this was not convincing, the ewe was left for a few more days with the ram after noting the date of the initial suspicion. About ten ewes were kept with the ram all the time, replacing bred ewes with new ones not bred.



To ascertain pregnancy and estimate the size of foetus each suspect ewe was subjected to radiography a few days prior to hysterotomy. Radiographic factors of 45 mas. and 50-60 Kvp, depending on the size of the ewe, were used, with the ewe laterally recumbent on her left side. This was an extremely accurate investigative procedure which saved unnecessary hysterotomy (Fig. 4), and has not hitherto been reported in this context.



Figure 4      Radiograph of Pregnant Ewe

## METHOD

### Preparation for Surgery

This was kept as simple as possible. The ewe was brought to the animal area at least 24 hours prior to surgery and water was withheld for 6 hours to avoid severe dehydration. Ninety minutes before surgery, the ewe was given premedication in the form of Chlorpromazine hydrochloride (either as Elmarine or Largactil whichever was available in supplies), intramuscularly, from 100 mg. to 200 mg. depending on size, the majority being given 150 mg. I.M. On two occasions, the intravenous route was used, giving 100 mg. This was found to cause a more severe hypotension, and a great deal more generalized oozing at operation. At the onset of our experiments, we used Perphenazine (Trilafon) 60 mg. (at an estimated dose of 1.5 mg./Kg body) intramuscularly. It was decided to change to a cheaper, quicker-acting and equally effective drug after the preliminary studies. Choice of perphenazine as well as chlorpromazine was made because of their antinauseant, antimetic effect as well as the atropine-like effect, in the hope that regurgitation would be minimal, vomiting absent and sedation adequate. However, the parasympatholytic effect is weak and excessive overdosage has to be given to produce severe effects. Hypotension is not so much a problem in low intramuscular dosage.

The ewe was shaved to provide an uncontaminated field on the right flank and along the lumbar vertebrae from L1 to S1. The



position of L2 was marked, as well as the L6/S1 interspace, with a line drawn from the iliac crest to L6/S1 space, using indelible pencil. The animal was positioned on its left side and secured with good-sized twine on all four limbs.

#### Anaesthesia

The choice of the mode of anaesthesia was dependent on the thesis that the least possible effect was desired on the foetus. Thus any anaesthesia given should not pass the placental barrier in large quantities. Epidural anaesthesia was chosen, supplemented by local infiltration of xylocaine.

The skin of the premedicated ewe was prepared with metaphen and alcohol and draped in the standard sterile fashion. With an assistant holding the animal in such a way that the lumbar vertebrae would be acutely flexed, landmarks were ascertained with the help of the previous marking and palpitation of the right iliac crest, locating L6/S1, and counting the vertebrae cephalad by palpitating the spinal processes of the vertebrae. The L2/L3 interspace is a good level to choose. A skin wheal was raised over a diameter of about 1 cm. at the chosen site, using 2% xylocaine with 1:200,000 epinephrine, delivered from a 2.5 cc syringe on a 25 guage 1 inch needle. The needle was then changed to a 25 guage 1.5 inch needle and inserted into the interspace so that the syringe was in the horizontal plane and the needle perpendicular to the back of the ewe. The xylocaine in the syringe was then delivered dropwise and at the same time the

needle advanced bit by bit towards the epidural space until the ligamentum flavum was encountered. The needle was now withdrawn and a few minutes allowed for the xylocaine to take effect (Fig. 7). The epidural needle was then inserted at the chosen interspace, with the bevel of the needle pointing cephalad. Once the needle entered the interspinous ligament the introducer was removed. A 5 cc. glass syringe was tested to ascertain free movement of the plunger in the barrel and, if necessary, both were moistened with sterile water. It was then attached directly on to the epidural needle with about 4 cc. of air in the barrel of the syringe. At this time the position of the syringe and needle in relation to the table, floor and back of the ewe were checked. It is important to have the needle and syringe parallel to the table top and floor and, at the same time, perpendicular to the vertebrae. The needle should enter the canal in a straight fashion to allow for accuracy, ease and safety. With the back of the right little and ring fingers resting on the ewe's lumbar vertebrae alongside the needle to steady the hand, the latter was advanced gradually with the remaining fingers of the right hand. At each advancement of 2-3 mm. the plunger of the glass syringe was gently but firmly pushed in with the left hand. While the needle was in the interspinous ligament, the plunger was seen to bounce back. As soon as the epidural space was entered, a click was heard as the ligamentum gave way and at the same time, the pressure in the syringe was released and the contained air entered the epidural



space, so that the plunger no longer rebounded on pressure. (Fig. 5)

The glass syringe was then changed for another one containing 10 cc. xylocaine with 1:200,000 epinephrine, specially prepared for epidural anaesthesia. An attempt was then made to withdraw fluid from the space. This should not yield any fluid unless the spinal canal or a blood vessel has been entered. In either case, the procedure was repeated at another vertebral level. If no fluid was forthcoming, the xylocaine was then injected over a period of 3-5 minutes into the epidural space.

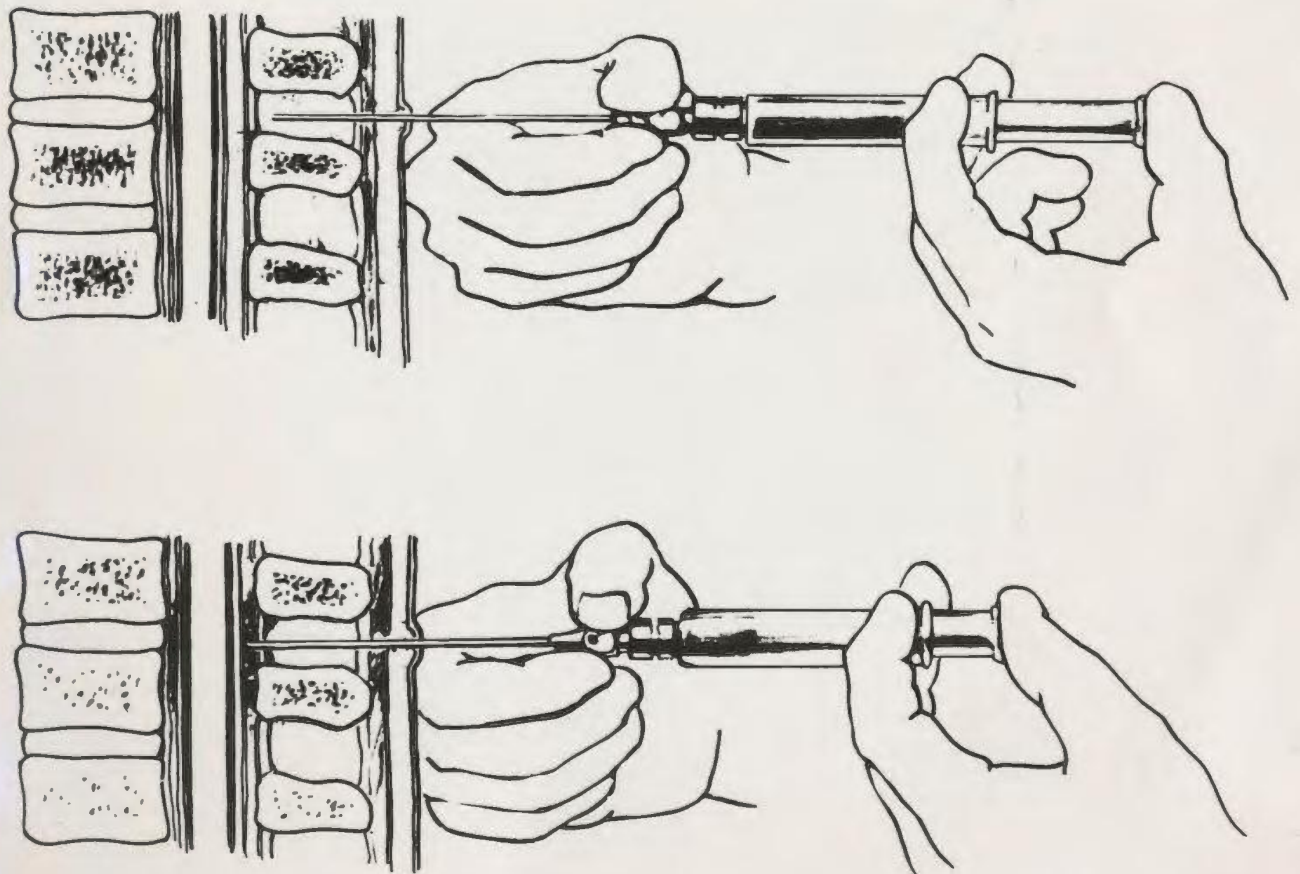


Figure 5 Placement of epidural space needle and injection of 10 cc. xylocaine with 1:200,000 epinephrine into the space.



Because of the late onset of action, which is between 10 and 20 minutes, local infiltration of the proposed site of skin incision was usually carried out with a further 5 cc. of 2% xylocaine and 1:200,000 epinephrine. This made it possible to start surgery with some confidence within 10 minutes of giving the epidural anaesthesia.

#### SURGERY

Two experimental procedures were followed. In one, Group A, the foetal lamb had its left ureter ligated. In Group B, the ureteral orifice was incised in an attempt to create vesico-ureteral reflux. In both groups the procedure was the same up until the foetus was located.

#### Group A- Ureteral Ligation

The ewe is placed on the left lateral position and each of its four limbs secured with ropes to the table. The back and right flank are cleansed in the usual fashion to assure sterility and draped, first for epidural anaesthesia and, after administration of anaesthesia, fresh drapes are applied to the flank for hysterotomy.

The effect of anaesthesia is tested on the site of the proposed skin incision and its adequacy confirmed. A transverse linear right flank incision is made about 15 cm. above the right iliac crest extending from a point about 10 cm. from the lumbar transverse processes for a length of 25 cm.

The skin wound is deepened to the muscle layer, securing bleeding vessels with haemostats along the way, to be ligated later.

Using a muscle-splitting procedure, the abdomen is opened and the abdominal viscera palpated.

The uterine horn containing the foetus is delivered and a section of the uterus is selected which will avoid damage to vessels and at the same time afford maximal exposure of the foetus. Babcock forceps are positioned about the site chosen so as to enclose it within an imaginary rectangle. The uterine wall is then incised, while an assistant keeps traction on it by holding on to the four Babcock forceps. The amniotic sac is next incised with a pair of scissors and the incision is enlarged to correspond with the length of the uterine incision. The position of each of the forceps is then changed so as to hold on to the edge of both the amnion and the uterine walls. With these forceps well positioned, the uterine horn can be controlled so that a minimal amount of amniotic fluid is spilt. Depending on the quantity of fluid in the amniotic sac, some of it is then aspirated into a sterile dish, or, if the procedure can be managed, without spilling a significant quantity, no fluid is aspirated.

The foetus is then brought out through the incision by holding on to the hind limbs and taking care not to rotate or twist the cord. The caudal end of the foetus is then placed on the uterine horn, which is covered with warm, moist sponges. The forceps are then crossed over around the foetus to avoid amniotic spillage, the foetus acting as a cork.



A vertical flank incision is now made in the foetus (which varies in length from 8 cm. to 16 cm.) extending for 1 cm. or thereabouts, parallel to, lateral and 5 mm. to the left of, the vertebral column. This incision usually extends from just above the left iliac crest to a few millimeters from the last rib. It is deepened using blunt fine forceps. The lower pole of the left kidney is identified and immediately below this the ureter is located running downwards and medially towards the bladder. Sometimes the ureter may be adherent to the peritoneum. It is then hooked round with a blunt curved fine haemostat and a 2-0 silk ligature placed around it, using a square knot.

#### Closure

A. Foetus: The foetal wound is closed in one layer using 4-0 silk on an atraumatic needle. Interrupted simple sutures are used. The closed foetal wound is sprayed with neosporin (a mixture of bacitracin, neomycin, and polymixin B) and the foetus then replaced in the amniotic fluid within the amnion. Any fluid previously aspirated is then restored, or if there has been a significant loss of fluid, warm sterile saline is added to the amniotic fluid to replace the estimated loss. 0.5 mega unit of Crystalline Penicillin in 1 ml. saline is added to the amniotic fluid before closing the amniotic sac.

B. Uterus: The uterus and amniotic sac are sutured in one layer using 3-0 chromic catgut on a running atraumatic needle. The uterine



wound is then inverted by running the suture line back over the incision to avoid spillage. The uterus is then replaced in the abdominal cavity and if possible, part of the omentum is brought down over the uterine wound.

C. Maternal Wound: The abdominal wound is closed in layers, using continuous chromic - catgut suture on an atraumatic needle. The skin is closed using 0 silk on a straight cutting needle. 0.5 mega unit Crystalline Penicillin in 1 ml. sterile water is given to the ewe intramuscularly.

Group B - Creation of Vesico-ureteral Reflux by Incising the Ureteral Orifice of the Foetus

The ewe is positioned, the skin prepared and draped as previously described for Group A and the same procedure followed up to the point of foetal delivery.

The caudal half of the foetus is now delivered while an assistant holds on to the four Babcock forceps, such as not to spill any amniotic fluid. These forceps are crossed around the foetus which then acts as a cork to prevent fluid loss. The hind limbs of the foetus are abducted so as to expose the lower anterior abdominal wall, as shown in figures 6 - 11. Warm moist sponges are then used to protect the foetus from loss of moisture and heat.

A vertical lower midline incision is made (a paramedian incision is made in the male to avoid the genitalia) and is deepened by blunt



Figure 6

Delivery of Uterus Into Operating Field

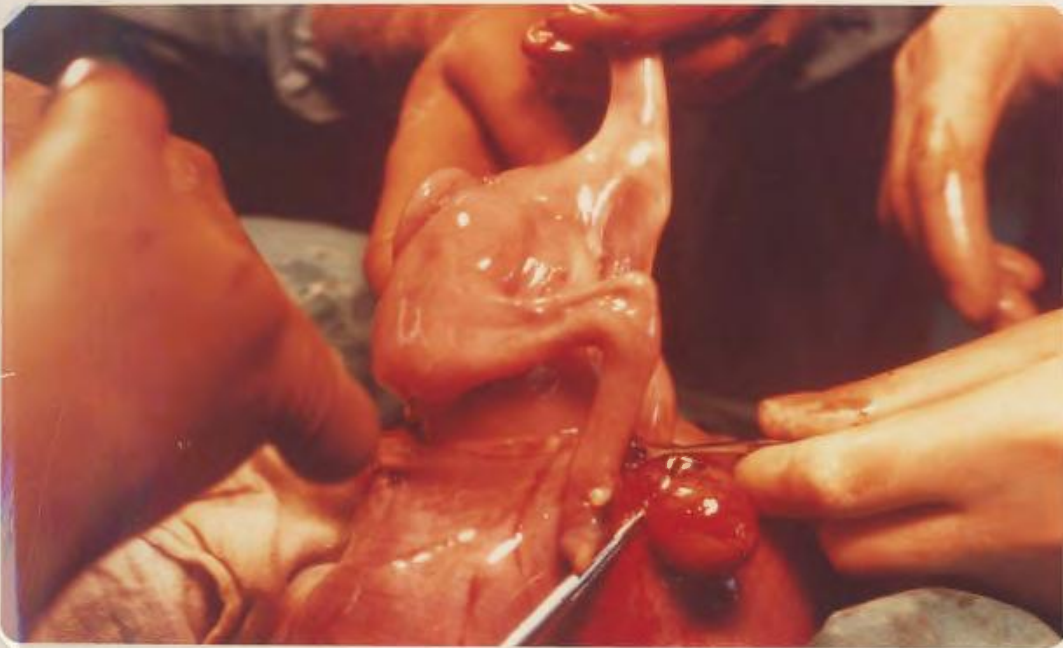
Note: (a) Moist Sponge on Tissue

(b) Babcock Forceps on Uterine Wall for Traction



Figure 7      Delivery of Foetus Into Operating Field





**Figure 8**      **Delivery of Caudal Half of Foetus Into Operating Field**  
**Note Crossover of Babcock Forceps to Prevent Spillage of**  
**Amniotic Fluid**



**Figure 9**      **Exposure of Foetal Urinary Bladder**

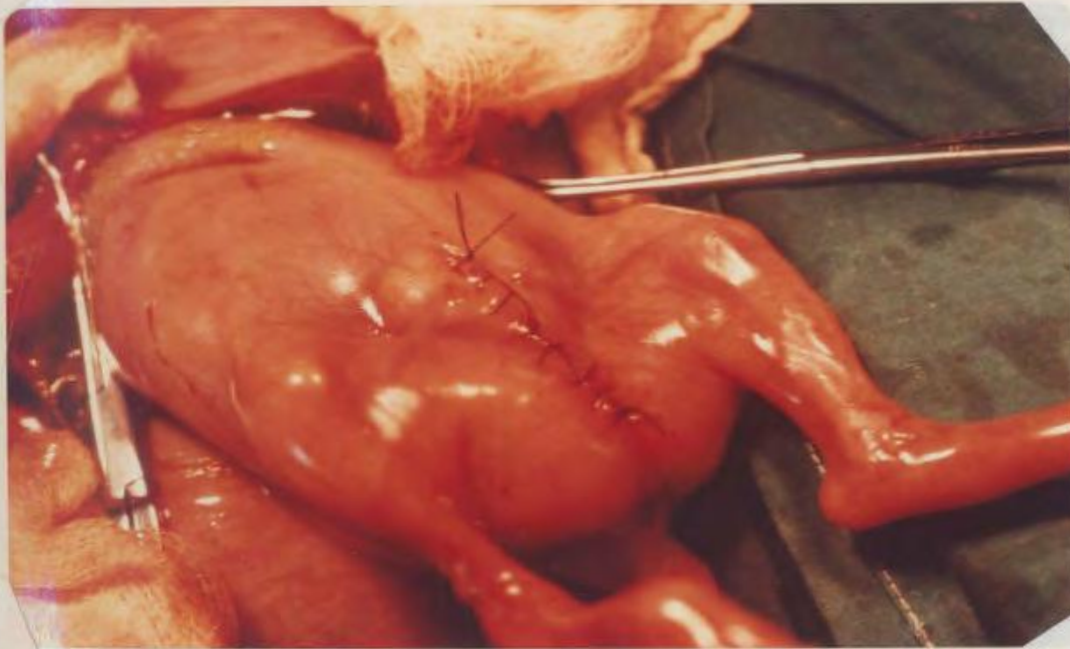


Figure 10      Closure of Foetal Wound Using Continuous Interlocking Sutures





Figure 11      Closure of Uterine Wound

dissection until the urinary bladder is located. Care is taken to avoid entering the peritoneal cavity at any stage of this procedure. The bladder is then incised anteriorly and vertically, each leaf of the bladder being held with a haemostat. The ureteral orifice to be incised is located with a fine hook and its roof incised in two places to create a form of triangular defect. The bladder is then closed with 4-0 chromic catgut.

### Closure

The foetal wound is closed in one layer with 4-0 silk on an atraumatic needle. The wound is sprayed with neosporin (bacitracin, neomycin and polymixin B) and the foetus is replaced in the amniotic sac. The amniotic fluid originally aspirated is replaced and any fluid loss is replaced by an estimated amount of warm normal saline. Experience showed that in this regard it is better to err on a low estimate than on a higher one, which might make uterine closure difficult and even hazardous. 0.5 mega unit of Crystalline penicillin in 1 ml. sterile water is instilled into the amniotic fluid.

The uterus, along with the amniotic sac, is closed in one layer, using a running chromic 0 catgut on an atraumatic needle. To ensure water-tightness, the incision is then inverted as described above. The uterus is replaced in the abdominal cavity and covered with the greater omentum.

The maternal abdomen is closed in layers with chromic 0 catgut on an atraumatic needle using running simple sutures. The skin is

closed using silk 0 suture on a straight sharp needle. The ewe is then given 0.5 mega unit of Crystalline penicillin in 1 ml. sterile water intramuscularly.

#### Post-Operative Care

The ewe is allowed to eat and drink as soon as it is placed in its pen and the disappearance of the effects of anaesthesia observed. Paralysis of the hind limbs usually lasts 3 - 4 hours, but occasionally lasts only 2 hours. Crystalline penicillin 0.5 mega unit is given I.M. to the ewe every day for the first 4 days post-op. The ewe is examined for evidence of vaginal bleeding, which is an early sign of imminent abortion. If abortion is threatened, no effort is made to prevent this, as it is an accepted sign of death of the foetus.

The ewe is discharged to the farm after completion of the course of antibiotics.



## RESULTS AND DISCUSSION

Twenty-six foetuses were operated on, of these three lambs were alive at full-term, but one of these died of atelectasis at the age of twenty hours. Ten were delivered at near term, but died shortly thereafter. Eleven foetuses were born macerated. Two sheep aborted spontaneously within two weeks of surgery; in both, unusually prolonged exposure of the foetus took place during intrauterine surgery.

It was not feasible to ascertain the cause of early intra-uterine death in the eleven foetuses recovered in a macerated condition. These were, however, transferred to the farm quite early in the post-operative period.

The ten foetuses delivered at near term would probably have been salvaged if our delivery conditions had been of a higher standard.

Of the three lambs delivered at full term, the one that died developed pulmonary symptoms of tachypnea, dyspnea, and shallow coughing, and was too sick to subject to radiographic studies before it expired. Post-mortem examination of the lungs revealed bilateral basal atelectasis with generalized bronchopneumonic process. No definite organisms were obtained on culture.

The two surviving lambs were studied radiographically and provided satisfactory evidence that the two types of procedures used had been successful. Lamb from sheep #35 had left ureteral ligation at 91 days gestation and was delivered at 140 days. Radiographically there was no excretion of contrast medium from the kidney above the

ligated ureter on intravenous pyelogram. (Fig. 12).

Lamb #37 had the reflux procedure performed at 90 days gestation and was delivered at 145 days. Cystography under fluroscopic control showed reflux on the operated side and this was recorded on film. (Figs. 13-14).

Post-mortem examination of the urinary tracts of the ten fetuses delivered near term revealed:

#### URETERAL OBSTRUCTION

##### A. Gross (Figs. 15-18)

1. Marked enlargement of the kidney above the ligated ureter, without any appreciable changes in gross vasculature. (Fig. 15).

2. Enlargement was proportionately related to the period of ligation.

3. Macroscopically on section the kidney with the ligated ureter showed marked hydronephrotic changes similar to the picture obtained in human hydronephrosis. (Fig. 18).

4. The ureter was markedly hypertrophied near the ligature, but thinned out as the renal pelvis was approached. (Figs. 15-16).

#### MICROSCOPIC

Technical difficulties were encountered in preparation of specimens suitable for histological examination. In error all specimens were preserved in 100% instead of 10% formalin. The mistake was discovered too late for anything to be done about it and the specimens were ruined for microscopy.



**Figure 12**      Intravenous Pyelogram (40 minute film) of a 24 hour Old Lamb With Left Ureteral Ligature. Note the Nephrographic Effect





Figure 13

Right Vesico-Ureteral Reflux - Intravenous Pyelogram Within  
Normal Limits on First Day of Life

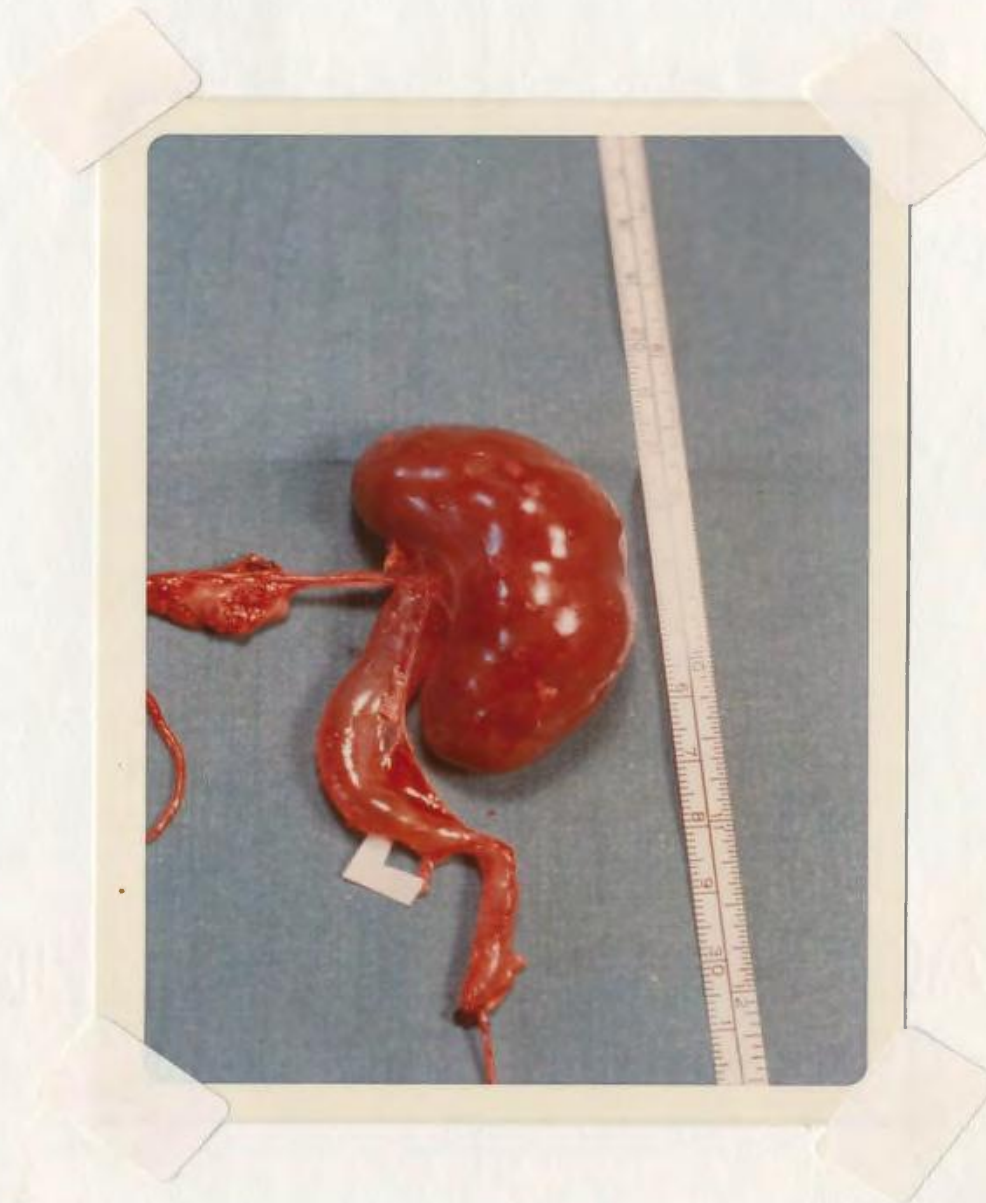


Figure 14      Right Vesico-Ureteral Reflux: Micturating Cystogram



**Figure 15**      **Left Ureteral Ligation: Fresh Autopsy Specimen.**  
**Note Gross Difference in Renal Size**





**Figure 16**      **Left Ureteral Ligation**  
                 **Left Renal Unit from Figure 17**  
                 **Note Thinning of ureteral Wall Near the Renal Pelvis**



**Figure 17**      **Left Ureteral Ligation**  
**Cut Sections of Left and Right Kidneys, Note Hydronephrotic**  
**Left Kidney**



**Figure 18**      **Left Ureteral Ligation**  
                     **Left Hydronephrosis**



The histological specimens, though spoilt, had sufficient detail to show a decrease in the number of glomeruli in the hydronephrotic kidney as compared with the contralateral side which was the control. Satisfactory illustration was not possible because of the formalin damage.

B. Reflux Procedure (Fig. 19)

The macroscopic picture obtained was unremarkable. There was no evidence of elongation, dilatation or tortuosity of the ureter. This might be explained on the basis of the short duration of the experimental process and, perhaps, if the reflux had been allowed to persist long enough the stigmata associated with this entity would be evident. The microscopic picture here was unremarkable partly because of the formalin destruction of the tissue, and also because of the short duration of the process.

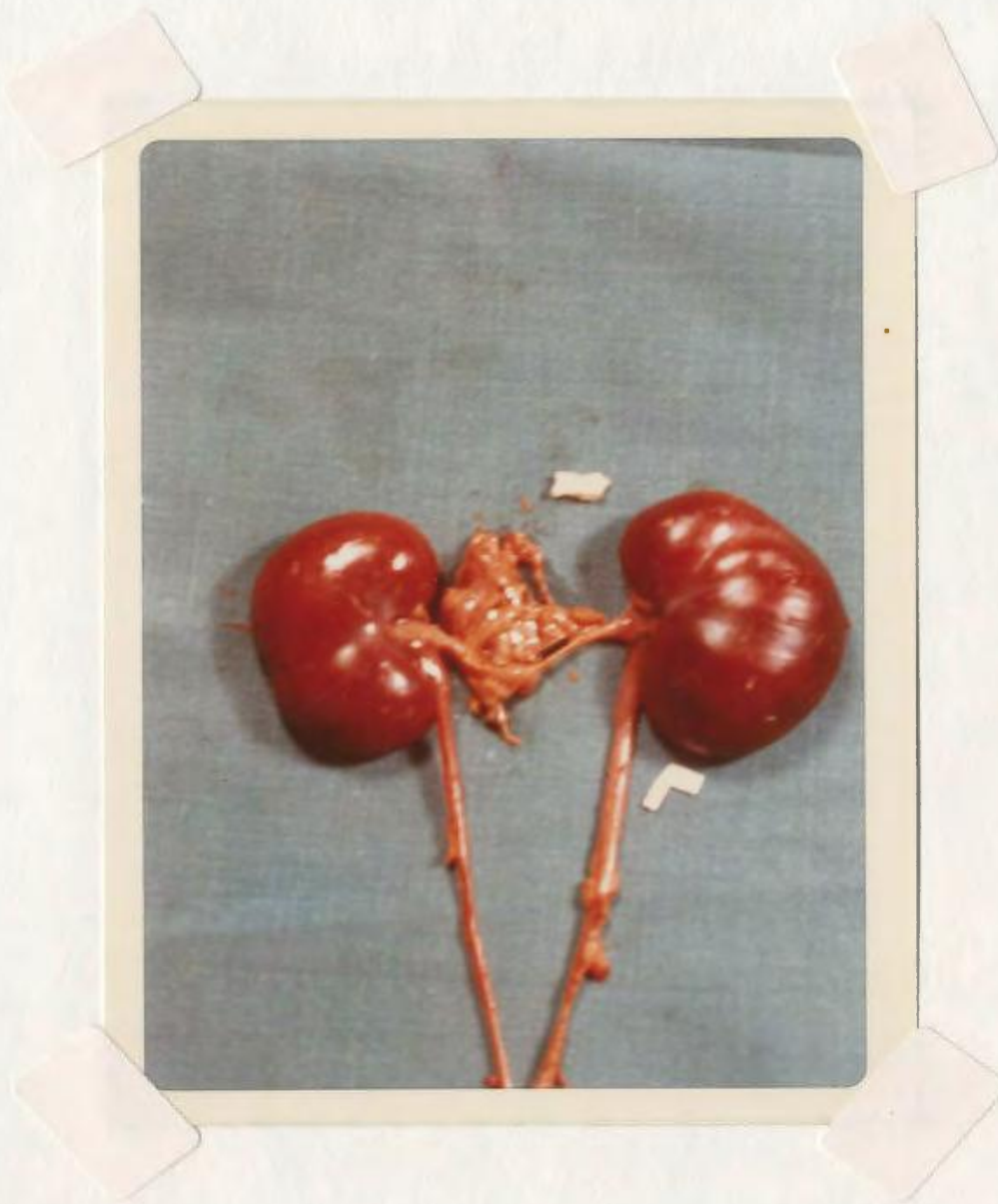


Figure 19 Left Vesico-Ureteral Reflux with Minimal Increase in Size of Kidney

## CONCLUSION

The experience gained in these experiments may be summarized as follows:

1. That the method of anaesthesia similar to that used by Beck, David A., presents a safe reliable anaesthesia in view of the absence of anaesthetic death or transference of anaesthetic agent to the foetus. Epidural anaesthesia, when properly administered, is the method of choice in these types of experiments.

2. That the real danger of amniotic fluid loss described in previous experiments can be avoided by the method adopted in our present series.

3. That the foetal tissues at this age are of a friable, gelatinous nature which requires meticulous care in suturing.

4. That the earliest practical foetal age is probably 70 days for operation type A (ureteral ligation) and 75 days for type B (reflux procedure).

5. That if the ewe is laid on her side, the danger from inhalation of vomitus appears to be negligible; although suitable preliminary pre-operative preparation may reduce the likelihood of vomiting.

6. That there is little or no immediate post-operative risk providing the procedure is carried out carefully.

7. That the fact of pregnancy and the size of the foetus can be estimated accurately by pre-operative radiography.



8. That there is no obvious factor which determines foetal death at a later stage, although minimizing the trauma of transportation of the ewe shortly after surgery may prevent subsequent foetal death.

9. That future work is required in this type of intrauterine surgery to obtain a statistically large yield, taking care to avoid pitfalls encountered in these early studies.

REFERENCES

- Mayer, A. Ueber den Einfluss des Eirstocks auf das Wachstum des uterus in der Fotalzeit und in der Kindheit und uber die Bedeutung des Lebensalters Zur Zeit der Kastration, Ztschrfd ges Gynak u. Geburtsh 77:279, 1915.
- Wolff, B. Experimentelle Untersuchungen uber die Entstehung extrauteriner Schwangerschaften und uber die Moglichkeit operativer Eingriffe beim lebenden Saugetierfoetus Beitr path. Anatomy 65:423, 1919.
- Nicholas, J.S.: Notes on the Application of Experimental Methods Upon Mammalian Embryos, Anal. Rec. 31:385, 1925.
- Nicholas, J.S. and Rudnick, D.: Development of Rat Embryos in Tissue Culture. Proc. Nat. Acad. Science 20:656, Dec. 34.
- Bors, E.: Die Methodik der intrauterinen Operation am uberlebenden Saugetierfoetus. Arch. Entw. D. Org. 105:655, 1925.
- Hooker, D. and Nicholas, J.S.: Foetal Cord Section, J. Comp. Neurology, 50:413, 1930.
- Tobin, C.E.: The influence of Adrenal Destruction on the Prenatal Development of the Albino Rat, Amer. J. Anat. 65:151, 1939.
- Jost, A.: On the Sexual Differentiation of the Rabbit Embryo. Remarks on the Subject of Certain Surgical Operations on the Embryo, Compt. Rend. Soc. de bid 140:461, 1946.
- Jost, A.: Experiences de decapitation de l'embryon de lapin. Compt. Rend. Acad. Sci. 225:322, August 1947.
- Wells, L.J.: Subjection of Foetal Rats to Surgery and Repeated Subcutaneous Injections: Method and Survival, Anat. Rec. 108:309-332, Oct. 1950.
- Louw, J.H. and Barnard, C.N.: Congenital Intestinal Atresia Observations on its Origin, Lancet 269:1065, 1955.
- Barnard, C.N.: A Method of Operating on Foetal Dogs in Utero. Surgery, 41:805, 1957.
- Barcroft, J. and Barron, D.H.: Genesis of Respiratory Movements in Foetus of Sheep, J. Physiol. 88:56, Oct. 1936.



- Coven, R.H. and Laurenson, R.D.: A Technique of Operation Upon the Foetus of the Rabbit, Surgery, 45:321, 1959.
- Jackson, B.T. and Egdahl, R.H.: The Performance of Complex Foetal Operations in Utero Without Amniotic Fluid Loss or other Disturbances of Foetal-Maternal Relationships, Surgery, 48:564, 1960.
- Blanc, W.A. and Silver, L.A.: Intrauterine Abdominal Surgery in the Rabbit Foetus: Production of Congenital Intestinal Atresia. Amer. J. Dis. Child., 104:548, 1962.
- Morgan, W.W. Jr., Rosenkrantz, J.C., Hill, R.B. Jr.: Hepatic Arterial Interruption in the Foetus - An Attempt to Simulate Biliary Atresia. J. Pediatric Surgery, 1:342, Aug. 1966.
- Rosenkrantz, J.C., Simon, R.C., Carlisle, J.H.: Foetal Surgery in the Pig with a Review of other Mammalian Foetal Technics, J. Pediat. Surgery, 3:392, June 1968.
- Beck, D.A.: Intrauterine Renal Surgery: Techniques for Exposing the Foetal Kidney During the Last Two-Thirds of Gestation. Investigative Urol., 8:2, 182, 1970.
- Beck, D.A.: The effect of Intrauterine Urinary Obstruction Upon the Development of the Foetal Kidney, Journal of Urology, 105:784, 1971.
- Barcroft, J., Barron, D.H., and Windle, W.F.: Some Observations on Genesis of Somatic Movements in Sheep Embryos, J. Physiol, 87:73, June 1936.
- Thomasson, B.H., Esterly, J.R., and Ravitch, M.M.: Morphologic Changes in the Foetal Rabbit Kidney after Intrauterine Ureteral Ligation, Investigative Urology, 8:261, Nov. 1970.
- Thomasson, B.H., and Ravitch, M.M.: Foetal Surgery in the Rabbit, Surgery, 66:1092, Dec. 1969.
- Osathanondh, V. and Potter, Edith L.: Development of Human Kidney as Shown by Microdissection III Formation and Interrelationship of Collective Tubules and Nephrons, Archives of Pathology, 76:290, 1963.
- Hodson, C.J.: The Lobar Structure of the Kidney, Brit. J. Urology, 44:246, 1972.





