SMITH, M.D., Blanca Povoli de, 1922—
PRE- AND POST-NATAL DEVELOPMENT OF THE
INTRAMURAL VESICAL GANGLIA IN CHILDREN.

The Ohio State University, Ph.D., 1964
Anatomy

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PRE- AND POST-NATAL DEVELOPMENT OF THE INTRAMURAL VESICAL GANGLIA IN CHILDREN

DISSERTATION
Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By
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* * * * * * *

The Ohio State University
1964

Approved by

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This work is dedicated
with admiration and gratitude
to
Ralph A. Knouff, Ph.D.
Professor Emeritus of
Histology and Embryology
ACKNOWLEDGMENTS

Dr. H. William Clatworthy, Jr., suggested the topic for this investigation and Dr. William A. Newton generously contributed the material and facilities of the Department of Pathology of the Columbus Children's Hospital for the conduction of this work. To both I am particularly indebted. Dr. J. P. Smith, Pediatric Urologist at Children's Hospital reviewed parts of the work and gave encouragement and advice for which I am grateful.

I am pleased to acknowledge the following persons for their assistance in the collection and preparation of the material for this work: Dr. Kazua Misugi, Mr. Louis Wensloff, and Mr. Tommy Yates, all from the Department of Pathology.

For assistance in illustrating this work I am especially indebted to Mrs. Carol Matz for the art work, Miss Margaret Nestor for the photography, and to Dr. Nobuhisa Baba and Mr. Robert Chamberlain for the microphotography.

Thanks are warmly extended to Miss Anne A. Warmington, Head, Children's Hospital Library, for her expert assistance in the surveying of the literature and to the Ohio State University Health Center Library staff for their cooperation.

A special mention of gratitude is made to Mrs. Melba Griffin, Ph.D. Counselor, Graduate School, who most significantly contributed to this work by her untiring guidance and encouragement.

And to Miss Coralei Betz, R. N., for her loyal support and devoted help.
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INTRODUCTION

In a previous study on the pre- and postnatal development of the enteric neurons in children, random examination of bladder sections suggested a more precocious development of the vesical ganglia. As a review of the literature failed to throw any light on the problem, the present investigation was undertaken.

The study was designed to document the morphological changes that the vesical ganglia undergo during growth and development from the second trimester of intrauterine life to the age of 14 years; and to correlate these changes chronologically with the maturation of the enteric neurons in the same subjects.

As the available information on ganglion cells of the human bladder is very limited, it became necessary to include in the study an investigation of the regional and histological distribution of the vesical ganglia. In view of the bilaterality in extrinsic bladder innervation it was deemed necessary to ascertain whether the intrinsic innervation of the two sides of the bladder is symmetric.

In addition, the vesical ganglia were studied in children with congenital anomalies of the pelvic organs or of its innervation pathways. The purpose was to determine whether the occurrence of those malformations may have altered the normal development of the vesical ganglia in utero.

As children born with those malformations often have later disturbances of bladder motility associated with chronic urinary infections it was thought of interest to determine whether the chronic infection per se affects the vesical ganglia.
To determine the nervous control of the bladder with its simple structure and its single function, might seem no hard task, yet some of the main facts are still disputed.

--Elliot, 1906

This brings up the question of how innervation of the bladder muscle is accomplished.

--Torbey and Leadbetter, 1963

The subject of bladder innervation was extensively studied between the latter years of the nineteenth century and the beginning of the twentieth century (Langley, 1895, 1896; Fague, 1902; Elliot, 1905, 1906; Fearnside, 1917). Although many investigators have since contributed voluminous additional data, the topic remains obscure. Discrepancies are particularly evident when correlations between morphological and functional studies are attempted. In the extensive literature review by Gruber (1933) several such instances are commented upon.

Present knowledge of the bladder development, structure, and autonomic innervation will be summarized under the headings of Developmental and Microscopic Anatomy of the Bladder, Extrinsic and Intrinsic Innervation. A brief review of comparative anatomy and physiology of bladder innervation are also included.

**Developmental Anatomy of the Bladder**

In human embryos of six somites (Arey, 1962) the caudal termination of the primitive gut forms a dilated blind sac, the cloaca.
On its ventral surface the entoderm is in contact with the ectoderm forming the cloacal membrane.

The cloacal membrane faces first ventrally and later, due to the growth of the tail, reverses so that it looks towards the attachment of the umbilical cord in the body wall. The mesoderm proliferating from the primitive streak passes around the cloacal membrane from either side and contributes to the formation of the lower anterior abdominal wall and genitalia. The cloaca will be split later by the urovesical septum into a dorsal rectum and a ventral urogenital sinus. The urorectal septum will reach the cloacal membrane at the primitive perineum during the seventh week. By then the urogenital sinus has the mesonephric or Wolffian ducts opening into it as they reach the lateral wall of the cloaca during the fifth week.

The ureteric bud originates also in the fifth week as an outgrowth from the dorsal wall of the mesonephric duct close to its entrance into the cloaca. It grows actively, at first dorsally, and then dorsally and cranially. This brings its tip into contact with the caudal undifferentiated mesoderm of the nephrogenic cord. This tissue forms a cap over the free somewhat dilated extremity of the ureteric bud, and it is termed the metanephric blastema. As the ureteric primordium elongates the blastemal cap is carried cranially upon it from the original position opposite the first and second sacral segments. The cranial tip of the ureteric bud by repeated subdivisions produces the collecting system of the adult kidney.

The site of entrance of the mesonephric ducts divides the urogenital sinus into an upper vesico urethral primordium and a lower
urogenital sinus proper. The cranial part of the vesicourethral primordium dilates to form the bladder. The terminal part of the mesonephric duct becomes incorporated into the lower part of the vesico-primordium so that the duct and the ureter acquire separate openings. The opening of the mesonephric duct, which becomes the ejaculatory duct, seems to remain stationary at the boundary between the urogenital sinus and urethra while that of the ureter is moved away cranially and laterally by growth changes at the base of the bladder. As these two openings migrate further and further from each other the mesodermal tissue between them becomes the vesical trigonus. Since the mesonephric ducts are of mesodermal origin, absorption of them into the bladder wall contributes a mesodermal component to an otherwise entodermal structure. The allantois also terminates in the cloaca and regresses as development proceeds. It ultimately becomes a fibrous cord connecting the apex of the bladder to the umbilicus.

Microscopic Anatomy

In view of the dual embryological origin of the bladder and the differences in microscopic anatomy and innervation between the detrusor and the trigonal musculature it seems warranted to describe them separately. The detrusor muscle covers most of the surface area of the bladder. It is characterized by its meshwork of muscle fibers which decussate several times in an orderly fashion (Campbell, 1963). The distensibility required to fulfill the reservoir function of the bladder is favored by a loosely attached submucosa rich in elastic fibers. The
transitional multilayered epithelium may flatten on distention of the bladder to almost a single layer of cells.

The trigonal muscle lies on the base of the bladder superimposed on the inner surface of the detrusor muscle (Hinman, 1935). The trigonal musculature has been shown to separate easily from the detrusor musculature by Mall in 1896. Young (1921), using Mall's technique, illustrated well the existent cleavage plane between the two muscle layers occupied by a layer of blood vessels. The region of the trigone due to the superimposition of the two muscle layers is the thickest in the bladder. Distensibility is minimal because the trigonal muscle is covered by a tightly adherent mucosa without intervening submucosa.

Bell in 1812 had shown that longitudinal musculature of each ureter was continuous with that of the trigone of the corresponding side. The fibers of Bell's muscles were described as extending fanwise from the ureteral orifices, some passing medially to make Mercier's bar or interureteric ridge, others to interlace with the corresponding ones in the middle of the trigonus and still others to extend into the wall of the urethra. The Bell's muscles of the two sides converge at the vesical orifice causing the trigonal musculature to be thicker at that point.

The trigonal muscle passes down over the posterior border of the vesical orifice and spreads out as an internal longitudinal layer over the posterior aspect of the urethra. The fibers pass between the opening of the prostatic ducts, ejaculatory ducts and prostatic utricle and continue down for some distance past the verumontanum. Some fibers may be traced as far as the beginning of the membranous urethra.
The external vesical sphincter made up of striated muscle fibers first appears as a few fibers very close to the urethral mucosa at a point opposite the mediolateral aspect of the vesical orifice. The fibers gradually increase in number passing farther and farther dorsally until they completely surround the urethra at a point opposite the apex of the prostate.

**Extrinsic Innervation**

The extrinsic innervation of the bladder depends upon the sympathetic, the parasympathetic, and the somatic systems. The sympathetic and parasympathetic nerves combine to form the pelvic plexus before reaching the bladder (Fig. 1). The pelvic plexus as described by David (1933) consists of interlacing groups of nerve fibers and of many small ganglia situated on either side of the ampulla of the rectum.

Ashley and Anson (1946) demonstrated in extensive dissections conducted on adult male cadavers that the pelvic plexus was located retroperitoneally in a parietal position in the dorsal portion of the pelvic cavity. These authors emphasized the fact that the plexus lies close to the bony pelvis instead of against the visceral wall as classically depicted. These authors also indicated that three nervous strata could be clearly identified at this level, the pelvic plexus occupying an intermediate position, deep to the hypogastric plexus but superficial to the sacral roots.

By far the largest sympathetic contribution to the pelvic plexus is from the hypogastric nerve, a direct continuation of the hypogastric plexus. The sympathetic fibers are joined by the parasympathetic nerves
FIG. 1

HYPOGASTRIC PLEXUS

VESICAL PLEXUS

PELVIC PLEXUS

PELVIC NERVES

PUDENDAL NERVE
originating from the second to the fourth sacral. The two systems blend into a single large flat band overlying the deep hypogastric vessels. The shape is that of a large Y, approximately 8 cm. long, the sympathetic forming the lateral arm, the parasympathetic, the medial. After the fusion of the two it continues as one large flat sheet composed of many nerve fibers and sheaths of connective tissue. To these two contributions, a few fibers from the pelvic chain ganglia are added. The main mass of the plexus courses in a curving line—concave anteriorly and the fibers reach the bladder, prostate urethra and anal canal as these rest upon the pelvic floor.

**Sympathetic components of the pelvic plexus.**—The efferent sympathetic fibers, as described by White (1935) are preganglionic fibers from lower thoracic and upper lumbar levels of the intermedio lateral columns. The axons of the neurons traverse the white rami of the lower thoracolumbar outflow. Some descend into the pelvis from abdominal level to form the mesenteric and hypogastric plexus where they synapse before reaching the bladder. Others have synapsed previously in ganglia of the celiac, renal, mesenteric or aortic plexuses. A few sympathetic fibers synapse in the last lumbar, and others in the sacral sympathetic chain.

**Parasympathetic components of the pelvic plexus.**—The five parasympathetic nerves first gather and then enter the pelvic plexus as a set of anastomotic nerves. Their cells of origin are in the intermedio-lateral columns of the sacral region. Of the five nerves, according to Ashley and Anson (1946), the cranial two arise from the junction of the
second and third sacrals; the third arises from the third sacral; the fourth from the fourth sacral entirely and the fifth from the junction of the third and the fourth. Although joining the pelvic plexus, the parasympathetic fibers are generally accepted to synapse only when reaching the ganglia of the bladder wall (Fig. 2).

**Somatic innervation.**—The somatic innervation is represented by the pudendal nerve which originates from the sacral roots 1 to 5 and contains both motor and sensory fibers.

**Central pathways.**—The center for urination is located in the preoptic region of the hypothalamus. The hypothalamus is recognized as the most important converging point of autonomic pathways but connections with the frontal cerebral cortex and the cingulate gyrus have been described (Langworthy, 1940; Simmons, 1940).

The exact pathway through the brain stem and spinal cord has not been definitely established at this time. But it is believed that a pathway leads backward from the hypothalamus and finally passes, perhaps, in the ventrolateral white column of the cord to end in cells in the gray column at the level of S₂, S₃, and S₄ (Denny-Brown, 1933; Van Duzen, 1953). The anatomical extent of these regions differs from individual to individual of each species, and more widely from species to species (Gruber, 1933).
INNERVATION OF THE HUMAN BLADDER

FIG. 2
Comparative Anatomy

Numerous studies on the autonomic innervation of the bladder in animals have contributed valuable information although often conflicting. That lack of uniformity exists between species in the anatomy and physiology of the urinary passages is well known.

The pelvic parasympathetic nerves are motor to the detrusor in all vertebrates and therefore are the main motor nerve of the bladder. Although it is generally agreed that the hypogastric nerve also innervates the bladder of vertebrates, Elliot (1905) demonstrated marked differences in the extent of the response to its stimulation, depending upon the animal used.

A comparative study by Elliot (1906) of the bladder and trigonus of dog, rabbit, cat, frog, and ferret pointed out that the area innervated by the hypogastric nerve is not necessarily confined to the boundaries of the trigonus (Fig. 3). In the frog and the toad both of those areas do not coincide as the bladder is a ventral outpouching from the rectum, while the ureters open independently into the dorsal surface of the rectum. Elliot demonstrated that mild contractions of the bladder extending into the fundus were obtainable on sympathetic stimulation although the bladder on these animals is purely cloacal without connections with muscle of the Wolffian type. Inhibitor fibers, to the fundus of the bladder only, have been demonstrated in the cat, while in the ferret they embrace the whole bladder.

As Gruber (1933) has emphasized the efferent nerves to the bladder vary not only in their origin and pathway, between different animal species, but also in different animals of the same species and
INNERVATION OF BLADDER AND URETHRA

FIG. 3

 AFTER ELLIOT, 1960
from left to right in the same animal. As a result the application to humans of the findings obtained in animal experiments regarding bladder physiology may be misleading.

In addition it must be mentioned that in regard to both contraction and relaxation, the type and depth of anesthesia has been found of decisive importance in the interpretation of results (Gruber, 1933).

**Physiology**

The detrusor and the vesical neck are under parasympathetic innervation. Pharmacologically this has been verified by the profound effect that cholinergic and anticholinergic drugs have upon contractions of the bladder (Fig. 4). Sectioning of the sacral or parasympathetic nerves produces relaxation of the internal vesical orifice and paralysis of the detrusor muscle. The fact that the trigonus contracts in treatment with epinephrin and responds to ergotoxin is pharmacological evidence that it is innervated by true sympathetic fibers (Wesson, 1920). Sectioning the hypogastric nerve causes paralysis of the trigonus (Van Duzen, 1953).

The trigonus is said to be devoid of parasympathetic terminals (Wesson, 1920). This conclusion is based on the negative response to phylocarpine and physostigmine. As the response to nicotine is positive indicating the presence of ganglia, it follows that from the pharmacological standpoint the ganglia of the trigonal area are sympathetic; but as will be commented later, there is no agreement as to the nature of the ganglion cells in the bladder.

For many years it was postulated that the two components of the autonomic nervous system had an antagonistic effect upon the bladder
SYMPATHETIC
• PREGANGLIONIC
• POSTGANGLIONIC

DETRUSOR

TRIGONE

C = CHOLINERGIC SYNAPSE
A = ADRENERGIC SYNAPSE

PARASYMPATHETIC
• PREGANGLIONIC
• POSTGANGLIONIC

FIG 4 AFTER NETTER, 1957
musculature and its sphincters. The dual antagonistic theory is no longer
tenable (Fague, 1902; Greene, 1963). Now it seems certain that the
bladder can empty exclusively by parasympathetic stimulation, and it is
generally accepted that the detrusor is the muscle responsible for
bladder emptying.

What remains uncertain is the extent of the participation of the
sympathetic system in bladder motility (Kuntz, 1944). Some authors
believe strongly that the sympathetic has no influence on the initiation,
maintenance, or inhibition of urination in human beings (Greene, 1963).
Others think that it may be responsible only for blood vessel innervation,
having no part on the sphincteric control or the motility of the bladder.
The participation of the sympathetic on urination cannot, however, be
entirely dismissed in view of the known activity of the trigonus during
urination (Fague, 1902). Young in 1918 first suggested that one function
of the trigonus was to pull open the interior sphincter of the bladder.

Bell in 1812 first demonstrated the anatomical continuity of
ureteral muscle fibers into the trigonus of the corresponding side and
their continuation into the urethra. Kalisher in 1900 first suggested
the anatomical distinction between the trigone and the rest of the
bladder. He inferred the dual ontogeny of bladder and trigone from the
contrast in the histology between the two structures. Langley and
Anderson (1895) had indicated that the innervation of the internal genital
organs is solely by sympathetic nerves and that cloacal derivatives have
a double autonomic innervation. Fagge (1902) also thought that the
ureters and urethra derived from the Wolffian duct were innervated
exclusively by sympathetics while the fundus of the bladder derived from
the allantois is innervated only by the pelvics. Tanagho and Pugh (1963) referred as "combined unit" to the continuous structure from the renal collecting tubuli in one side, to its ureter, half of the trigone, and Bell muscle fibers to their termination into the urethra. These authors stated that the prevention of reflux of urine from the bladder into the ureters during micturition was dependent upon the integrity of the "combined units."

The innervation of this combined unit is sympathetic and contraction of the distal ureter, trigonus, and urethra are demonstrable on stimulation of the hypogastric nerve of the ipsilateral side.

The phases of urination have been variously described. The most accepted sequence is based on the mechanism described by Young and Macht (1924); also supported by Van Duzen and Duncan (1953). The phases are as follows: an initial contraction of the trigonus which results in a depression of the posterior lip of the internal vesical orifice; then a wave of contraction in the detrusor musculature starting in the region of the internal vesical orifice and extending backwards towards the dome of the bladder, this further pulls on the internal vesical sphincter keeping it open. The last phase of urination consists of contraction of the abdominal musculature resulting in an increase in intrabdominal pressure.

**Intrinsic Innervation**

Ganglion cells in the bladder wall of mammals were first described by Remak in 1840. The majority of studies have been conducted in animals (Darwin, 1874; Michailow, 1908; Iljina, 1932; Polykarpowa, 1935; Moseley, 1936; Kuntz, 1936) and few descriptions are available of the human vesical ganglia (Stöhr, 1928; Leibowitz, 1963).
During the last thirty years there has been considerable interest in the ganglion cells of the enteric plexuses in man and a vast amount of literature has been contributed by authors from all over the world. Progress on the knowledge about the vesical ganglia has lagged behind; they are rarely mentioned in the current literature and reference to them is frequently absent from recognized textbooks in the related fields.

**Embryogenesis of vesical ganglia.**—While the embryogenesis of the enteric neurons has been extensively investigated, that of the neurons of the vesical ganglia has attracted little attention.

The point of view that most, if not all, of the ganglion cells of the sympathetic trunk are derived from the neural tube is supported by Kuntz (1922), Brizzee (1949), and most recently Raybuck (1956). That the neural crest is the exclusive source of enteric neurons is the opinion held by most authors. Among them are Muller and Ingvar (1923), van Campenhout (1932), and Yntema and Hammond (1945).

The 1953 report of Yntema and Hammond following extirpation of the vagal neural crest in a chick embryo of 7 to 10 somites is of special interest because it pointed to a differential level of origin for the enteric and the vesical neurons. The experiment resulted in an aganglionic segment of the distal bowel, but the sympathetic and pelvic ganglia developed normally. If the neural crest of the trunk was extirpated instead, the pelvic ganglia failed to develop while the intramural ganglia of the distal bowel were normal.

It has been reported that enteric neurons can differentiate even in the absence of preganglionic neurons (van Campenhout, 1932; Keuning,
Similar work has not been done with vesical neurons. Neither are data available regarding the possibility of neurons arising locally as Tello (1924) postulated for the enteric neurons.

**Nature of the vesical ganglia.** Among the many obscure issues in bladder innervation, the question as to whether the vesical ganglia are sympathetic or parasympathetic has received little attention.

As it is classically described and generally accepted the pre-ganglionic fibers of the sympathetic system synapse in ganglia external to the viscera while the parasympathetic ones penetrate the viscera to synapse. The bladder, however, seems to present an exception to this rule as Kuntz (1957) describes sympathetic and parasympathetic preganglionic fibers synapsing in ganglia of the pelvic plexus as well as in ganglia within the bladder wall.

While in the enteric plexuses it is agreed that the ganglia are entirely parasympathetic, those of the bladder are variously considered by different authors. Stöhr (1932) states that they are sympathetic but Iljina and Lawrentjew (1932) in an experimental study in the dog concluded that intramural ganglia of the urinary bladder are exclusively parasympathetic. This view was supported by Polykarpowa (1935).

Based upon an experimental analysis of the ganglion cells in the cat's bladder Kuntz and Moseley (1936) reported that vesical ganglia were both sympathetic and parasympathetic; some receiving preganglionic fibers exclusively via the pelvic nerve; some either exclusively or at least mainly via the hypogastric nerve, and some via both these nerves. Their experiment consisted of severing the pelvic nerves by sectioning the
sacral nerve roots distal to the spinal ganglia. They observed fiber
degeneration in some of the ganglia, but not in others. Similarly when
the inferior mesenteric plexus and the sympathetic trunks from the second
lumbar segment caudalwards were sectioned, some ganglia showed pregangli-
onic fiber degeneration while others did not. In basis of this study,
Kuntz and Moseley estimated that 40 percent of the vesical ganglia were
sympathetic, 40 percent parasympathetic, and 20 percent mixed.

These authors also stated that at level of the trigonus the
ganglia were 50 percent sympathetic and 50 percent parasympathetic but on
pharmacological testings as previously discussed (p. 13) the trigonus is
said to be deprived of parasympathetic terminals. This discrepancy further
illustrates the difference of opinions regarding the nature of the vesical
ganglia.

**Morphology and distribution of ganglia.**—While in the bladder of
the frog as many as six different types of neurons have been described,
those of the human bladder are generally described only as multipolar.
During childhood, however, Stöhr (1928) reports two morphological differ-
et types, a large and a small cell type, and mentioned that several
transitional forms may be encountered. Illustrative drawings of the
vesical ganglia in man can be found in the German literature, mainly in
the works of Stöhr. In an extensive review of the English literature no
publication illustrated with microphotographs of human vesical ganglia
could be located.

Although most authors limit the presence of vesical ganglia just
to the adventitia, Muller (1924) reported them near the mucous membrane,
and Stöhr (1928) describes several scattered small ganglia in the muscularis. This latter author denies ever seeing them in the mucosa.

Kuntz and Moseley (1936) following their nerve degeneration studies in the cat concluded that ganglia located superficially are larger and parasympathetic in nature, while those located deeper within the muscularis are of smaller size and sympathetic. To this point we shall refer later when our findings are presented.

**Maturation of autonomic neurons.**—De Castro (1932) demonstrated that in the ganglia of the vertebral and paravertebral chains of the human fetus the neurons did not develop simultaneously but gradually followed a distinct developmental sequence. The work of Kuntz in 1938 dealing also with the sympathetic ganglionated chain supported de Castro's view. De Castro was one of the first to postulate that the autonomic ganglia may undergo continuous growth and differentiation throughout life. Figure 5 illustrates the accepted progression in maturation of the neuron, from the apolar neuroblast to the multipolar mature neuron.

The maturation process of the neuron (Fig. 5) consists of an increase of cell size, principally because of an increase in cytoplasm and Nissl's substance, a sharper nuclear membrane, and the presence of nucleoli. There is also evidence of growth in the cell prolongations, axons, and dendrites. The ganglion cell is outstanding for its very high metabolism and its relatively high content of RNA which determines its pronounced basophilia.
MATURATION OF THE NEURON

EDULLARY EPITHELIUM

MEDULLOBlast

APOLAR NEUROBLAST

BIPOLAR NEUROBLAST

UNIPOLAR NEUROBLAST

MULTIPOLAR NEUROBLAST

NEURON

FIG. 5
In the U. V. microspectrographic analysis popularized by Hyden for study of the nerve cell composition it was demonstrated that the protein substrate in the cytoplasm increases more than 2000 times during the development from neuroblast to the adult cell with completed growth.

Developmental changes on the ganglia of the enteric plexuses of children have been reported by Friesen (1956) for the pylorus and by Smith (1960) for the rectosigmoid. Studying the development of the regulatory centers for water metabolism in newborn infants, Rodeck (1959) describes a similar progression in the ganglion cells of the supra-optic and paraventricular nuclei of the hypothalamus. Gapeen (1963) and Korochkin (1963) have recently presented additional data on the developmental aspects of the enteric plexuses in man. Data on the pre- and postnatal development of the vesical ganglia in man are not available.
MATERIAL AND METHODS

Material

The material was obtained from the Departments of Pathology of the Columbus Children's Hospital and The Ohio State University.

The material consisted of sections of urinary bladder in 85 children and it was divided into three groups:

Group A: The bladder and ureters of 2 newborns; one normal and the other with agenesis of the kidney and ureter on the right side.

Both specimens were obtained at autopsy.

Group B: Blocks from the ureterovesical junction in 47 children. In 30 of the 47 cases the cardias, pylorus, and distal 10 cm. of the rectosigmoid were also obtained for comparison.

The age distribution of the patients was as follows:

Prenatal: 15 cases; 6 fetuses from the second trimester,
9 fetuses from the third trimester of gestation.

Postnatal: 32 cases whose age distribution was as follows:

Neonatal, birth to 1 month, 9 cases
Infancy, 1 month to 2 years, 8 cases
Early childhood, 2 to 6 years, 8 cases
Late childhood, 6 to 14 years, 7 cases

Specimens were obtained at autopsy. Included were only children dying of a brief non-infectious illness or an accident and not
having any known or demonstrable pathology of the urinary tract, central nervous system, or pelvic organs.

Group C: Bladder sections of 36 children ranging in age from birth to 16 years of age. Half of the 36 cases had congenital malformations without urinary tract infection. Malformations of the urinary tract, the rectum and anus, the abdominal parietes and the neuroaxis were represented in the group. The remaining half had chronic urinary infections, and in all but four the infection was superimposed on a congenital malformation of the urinary tract.

Twelve of the biopsies in Group C had been obtained by open operation on the bladder without resourcing to electrocoagulation and the remaining at autopsy.

Method 1 (used in Group A, cases No. 1 and No. 2)

The bladder was injected in situ with 10 percent formalin and removed in continuity with the lower ureters and the urethra. Following fixation in the distended state, the bladder was bisected and blocks cut. The blocks on one half of the bladder were cut longitudinally and those of the remaining half sagitally (Fig. 6A).

The blocks were serially sectioned at 10 micra. Following the technique recommended by Leibowitz (1963) two consecutive sections in each millimeter were mounted and stained, one with hematoxilin and eosin, and the other with Gallocoyanin (Einarson, 1932). A second set of slides was prepared and stained with the cresyl violet-Luxol Fast Blue method of Kluver-Barrera (1953), combined with the silver method of Bodian (1937).
NORMAL DISTRIBUTION OF VESICAL GANGLIA

FIG. 6
Method 2 (used in Group B, cases No. 3 to No. 49)

At necropsy the bladder and both lower ureters were removed. Prior to fixation the bladder was opened and stapled over cardboard to avoid curling. The cardias, pylorus, and rectosigmoid specimens of cases No. 3 to No. 33 were equally handled. Formalin 10 percent was the fixative used in all cases.

From the fixed bladders blocks, one centimeter in length, were cut parallel to the interureteric ridge and including the ureterovesical junction. From the gastrointestinal tract ten blocks parallel to the lumen of the bowel were cut in each case; eight from the rectosigmoid, one from the cardias, and one from the pylorus. The eight rectosigmoidal blocks were cut at the following levels from the anal verge: one, two, three, four, five, six and a half, eight, and nine and a half centimeters.

The bladder and gastrointestinal blocks were serially sectioned at thickness of five micra, five micra, and ten micra. The serial sections were stained with hematoxylin-eosin, Kluver-Barrera, and Bodian-Kluver-Barrera, in that order. Additional non-serial sections were stained with Gallocyanin and with Kluver-Barrera-Holmes silver (Margolis, 1956).

Method 3 (used in Group C, cases No. 50 to No. 85)

Hematoxylin-eosin stained sections from the collection of the Department of Pathology, Children's Hospital, Columbus, Ohio. Additional sections were cut from the original blocks at 10 micra and stained by the same methods as the previous cases.
Regional Distribution of Vesical Ganglia

To determine the elective site for bladder biopsy, it was important first to investigate if the number and morphology of the ganglia and of the ganglion cells varies appreciably with the site biopsied. Similarly if the direction of the section influences results. Towards this purpose the bladder of a newborn male infant (case No. 1) was fixed in distention and later bisected. The blocks on one half of the bladder were cut longitudinally and those of the remaining half sagitally (Fig. 6A). On the stained sections from each block the number of ganglia present and of the cells contained therein were counted.

In the hemibladder cut sagitally we counted as many as 1000 ganglion cells in a single section from the blocks transsecting the base of the bladder, as shown in Figure 6B. The cells were still numerous in sections from the next two blocks, comprising the region of the trigon, but decreased gradually to only two or none per section from the subsequent blocks taken from the fundus of the bladder. On the hemibladder cut longitudinally the higher counts were in sections from its lateral aspect particularly at the entrance of the ureters where approximately 700 cells are seen in a single section. The concentration decreases anteriorly to about 200 or less per section.

Comparing several longitudinal sections from the region of the base of the bladder, the maximum concentration was seen at the ureterovesical junction as shown in Figure 6C. The study of the longitudinal
sections of the bisected bladder demonstrated that the number of ganglia decreases sharply from the base of the bladder towards the fundus and also that it decreases towards the midline anteriorly. Both hemibladders showed a paucity of ganglia in the region of the fundus.

**Histological Distribution of Ganglia**

*in the Bladder Wall*

(Illustrated by Plates 1 to 4 in the Appendix)

In our material we have observed that ganglia predominate in the superficial aspect of the muscle layer although some may lie partially within the adventitia. They may have as few as two ganglion cells or as many as a thousand. In the great majority of sections when ganglia are found with relative ease in the superficial musculature they are also found in appreciable numbers in the deeper muscle planes. Those on a deeper location commonly have between two to ten ganglion cells.

With less frequency but by no means rare is the occurrence of ganglia immediately adjacent to the mucosa, as shown in Plate 4. While ganglia were found with the greatest of ease in sections from the base of the bladder, it was difficult to find ganglia in sections taken from the fundus of the bladder; those seen in sections from the fundus and top of the bladder were frequently located deep in the musculature.

**Symmetry of Vesical Ganglia**

This was studied by comparing the distribution and morphology of the vesical ganglia in two bladders, both of new born male infants. One bladder was anatomically normal, case No. 1 previously described, and the other abnormal, case No. 2, with agenesis of kidney and ureter on the
right side. The kidney and ureter on the opposite side were normal although the kidney was slightly hypoplastic.

Both bladders were fixed in distention and bisected. On inspection of the interior aspect of the right hemibladder in case No. 2, there was no ureteric opening at the usual location and the normal outline of the trigonus was absent. Blocks were cut in the same manner in both bladders as shown in Figure 6A. This method allowed comparison of both sides of the same bladder as well as the normal with the abnormal bladder.

The findings in regards to the number of ganglion cells counted in the anatomically normal bladder have been previously given under the heading of Regional Distribution of Vesical Ganglia. As shown in Figures 6B and 6C counts approximated closely in the two sides as did the regional distribution. Morphology of ganglia and of ganglion cells was also normal and similar in both bladder halves.

In case No. 2, however, the findings were strikingly different in the normal than in the abnormal side. As Figures 7A and 7B indicate there was a paradoxic increase in the number of ganglia on the abnormal side, i.e., the side of the renoureteric agenesis. The maximum concentration of ganglion cells was observed in the sections from the block corresponding to the site where the ureter opening should have been.

In addition to the numerical difference, marked morphological differences were observed. Ganglion cells on the abnormal side showed definite evidences of degeneration affecting over 80 percent of the cells counted. Plates 5 to 12 in the Appendix illustrate those changes. On
ASSYMETRY IN VESICAL GANGLIA

FIG. 7
Silver stains, by the Bodian and Holmes methods, a normal compliment of preganglionic fibers were observed in the affected ganglia. If the number of postganglionic fibers within the ganglia were diminished, it could not be ascertained.

Pre- and Postnatal Development of Vesical Ganglia

Frenatal period

Observations for this period are based on the cases listed in Tables 1 and 2. The developmental progression is illustrated in Plates 13 to 28 in the Appendix.

Second trimester of gestation.—The bladder musculature during the second trimester is well defined but the muscle layers are separated from each other by wide open spaces filled by a loose connective tissue matrix. Although the ganglia are not well defined at this stage, one observes between the muscle bundles areas which in general conform to the shape of the future ganglia. Some of these areas are poorly defined, outlined just by the divergent direction of the muscle fibers, others seem to show a faint suggestion of a capsule; others show a better defined capsule and some are already densely populated by tightly packed early neuroblasts. Ganglia are distributed throughout the bladder wall and many are seen in the submucosa. The most superficial ones seem to get their quota of neurons earlier than the deeper ones.

Comparing the six cases in the group it is evident that the maturation of neurons is progressing actively from the third month, represented by case No. 3, to the sixth month, represented by case No. 8.
Cases No. 3 and No. 4, representatives of gestations of three to four months, show an overpopulation of their ganglia with early neuroblasts. The intense basophilia of the young neuroblasts makes them easily identifiable against the pale background obtained with a Galloycyanin stain of pH 1.6.

The nuclei stains darkly and there is only a thin rim of cytoplasm around it.

During the early part of the second trimester, as observed in cases No. 3 and No. 4 weighing 20 and 28 grams, respectively, the neurons are quite similar to those observed on sections from the enteric plexuses of the same subjects.

During the later part of the fourth month, however, there is a drastic change in the morphology of the vesical neurons and from that moment on there is no parallelism between the maturation and development of the vesical and that of the enteric neurons. This is particularly well illustrated by case No. 5, weighing 200 grams, and whose vesical ganglia are outstandingly different than those of the previous cases. The cells show a marked increase in the amount of Nissl substance. This is seen in several ganglia in the adventitia and muscularis. There is no actual increase in the number of ganglia seen or in the number of ganglion cells within ganglia; the startling effect of the "coming-of-age" of the neurons results from the changes in each individual cell. The nucleus remains slightly dark and the total size of each cell is still far less than those of older children, but for the first time the cells resemble typical ganglion cells. The maturation has not affected equally all ganglia and some seen deep in the muscle or in the submucosa are but
slightly changed from those seen in cases from the early part of this period. This would confirm the impression gained from the previous cases that the maturation is more precocious in the ganglia located in the adventitia.

A critical comparison between the adventitial ganglia of this and the earlier cases permits to be certain that the ganglia are similar in all respects and that the change is limited only to the transformation of early neuroblasts into larger, more mature cells. The excessive clustering of cells previously noted is also becoming less evident.

Third trimester of gestation.--Changes during this period are more evident in the nerve plexus of fibers and in the capsule of the ganglia than in the ganglion cells themselves, although the previous trends continue.

In the ganglion cells the nucleus is more often sharply delimited and near the eighth month the nucleolus has become a permanent component of the cell. The nucleus to cytoplasm ratio keeps on increasing gradually. The relationship of intramural bundles of nerve fibers to capillaries, characteristic of the bladder in older children, is starting to become apparent.

There is gradual progress also in the development of a capsule, comparing with the previous younger subjects. Capsules are not yet uniformly seen, however. The oval shaped ganglia in the periphery seldom have any at this stage and the effect is one of compression against neighboring muscle bundles. On round ganglia, often seen deeper in the bladder wall, perhaps because they are part of a nerve bundle, a better
defined capsule is seen. Nuclei of Schwann cells are possibly present in some of them.

Polarity of the ganglia is observable for the first time in this period. The ganglia adopts a comma shape disposition with an open end through which the entry or exit of fibers take place. If those fibers are pre- or post-ganglionic, they cannot be ascertained in this study. From the peripheral ganglia streams of nerve fibers accompanied by ganglion cells penetrate the bladder wall. They are best seen on silver impregnated sections.

On silver impregnation a network of thin fibers is seen in between the muscle layers but it is difficult to draw conclusions as to their nature. Many long processed, small bodied cells are seen in the spaces between the muscle bundles but it is still more difficult here than in the adult bladder to decide if they are connective tissue cells or the interstitial cells of Cajal. Bundles of nerve fibers are seen deeply into the bladder wall, some included into the submucosa.

In the remaining subjects representative of this period the above characteristics become gradually more pronounced. The chief indication of growth seems to be the increment on the amount of Nissl substance in each ganglion cell and the decrease in the degree of over-population of the ganglia. It must be stressed that it is still possible in cases of the sixth month of gestation to identify characteristics which were prevalent in cases of the third to the fourth month.
Postnatal period

Observations for this period are based on the cases listed in Tables 3 to 6. The development of vesical ganglia in this period is illustrated by Plates 29 to 40 in the Appendix.

In cases No. 18 to 26, newborns during the first month of life, the progression of development of the vesical ganglia is seen to continue gradually. Ganglion cells have increased in size and completely differentiated cells are seen more often. The larger neurons exhibit a sharper nuclear membrane and some also a well defined nucleolus.

More cells of the mature type are seen at this stage on a section from the bladder than in one from the pylorus or the rectosigmoid from the same subjects.

In cases No. 27 to No. 34, representatives of the 1-month to 2-year group, the trend seems to be toward complete filling of the ganglia by larger neurons with abundant cytoplasm and sharply defined nuclei. In silver stains the number of bipolar neurons have decreased considerably giving way to multipolar cells although not yet of full maturity size.

In cases No. 35 to No. 42, two to six year olds, the ganglion cells have a more faceted appearance than was seen in the earlier periods. This is probably caused by the presence of many more larger cells within the ganglia with resultant impingement of one upon another. Many nuclei of satellite cells are seen interspersed with the normal appearing ganglion cells. In silver stains the multipolar cells appear on the whole larger and with more complicated dendritic apparatus than in the previous periods.
In cases No. 43 to No. 49, of the 6 to 14 year age range, the differences with the preceding period are mostly related to the smaller neurons. Previously ganglia were populated mostly by monopolars and bypolars although multipolars were present in variable and gradually increasing numbers. In this period of late childhood, the reverse seems to be the case; multipolar neurons predominate although bypolars are still occasionally seen.

**Vesical Ganglia in Congenital Malformations**

Eighteen patients were studied in this group ranging in age from one day to four years (Table 7). Observations are illustrated by Plates 41 to 44.

None of the eighteen patients in this group has history of urinary tract infection or evidence of it at autopsy. Five patients had urinary tract anomalies (posterior urethral valves); two patients had deficiency of the abdominal wall musculature; three patients had anorectal malformations and eight patients had lumbar or lumbosacral meningomyelocele.

Observations failed to show any demonstrable abnormalities in the vesical ganglia. As these were random biopsies taken during routine autopsies from an undetermined site in the bladder no conclusions can be drawn as to the number of ganglion cells. In some of the sections ganglia were more readily found than in others. This is probably due to the former being from the base of the bladder and the latter from the fundus.
The morphology of the ganglia correlated well with the normals for the age of the patient as described in pages 35 and 36 of this study. On the galloycyanin stained sections Nissl substance appeared normal in amount and distribution. On the silver stained sections the nerves and nerve fibers in the ganglia were also of normal appearance and distribution.

The Effect of Infection upon Vesical Ganglia

This was studied in eighteen cases of which all but seven had also a congenital anomaly of the urinary tract (Table 8). Some of the anomalies were of the same type studied in the previous group but those patients had no urinary infection. It was felt that this method of selection would permit comparison of the vesical ganglia in patients with similar congenital malformations with and without infection.

Patients in this group ranged in age from four days to sixteen years. Twelve of the specimens were surgical biopsies obtained without the use of electrocoagulation. The remaining six were obtained at post-mortem.

Observations in this group are illustrated in Plates 45 to 52. In contrast to the rest of the study considerable difficult was experienced in this group to identify the vesical ganglia. In the patients from previous groups, ganglia were located easily by screening the microscopic sections at low power in either the H-E or the galloycyanin stained sections.

In patients of this group in either surgical biopsies or necropsy material, the galloycyanin sections were essential for locating the often
minute dark clusters of distorted ganglion cells. While in the rest of the study vesical ganglia presented with well delimited boundaries, the opposite was true in this group.

On galloccyanin stained sections the ganglia of patients in this group could be said to represent one of two general types: (1) normal shape but markedly reduced in size; (2) larger size and totally invaded by satellite cells with only one or two ganglion cells remaining.

The involvement of the ganglia becomes much the more striking when the size of the ganglia seen is compared with the average normal size for these structures in children of similar age (Plates 29 to 40).

The changes seen are not those of degeneration in individual ganglion cells but of involvement of the whole ganglionic structure.

No attempts at correlation were made between the bacterial agent incriminated in the urinary infection and the morphology of the ganglia. As cultures of the urine in most patients in this group had grown different organisms at different times it would have been impossible to properly assess the effect of any one of them.

Another characteristic observed on the sections of urinary bladder in patients with chronic urinary infection was the marked increase in the number and size of nerve fibers seen occupying an unusually superficial position in the bladder wall.

It was of additional interest to compare the appearance of the vesical ganglia in some of those patients with the enteric ganglia in
sections of their gastrointestinal tract. This comparison showed that the morphology of their enteric ganglia was within the limits of normality for the age established in a previous study (Smith, B., 1960).
DISCUSSION

The observations in this study concentrated on the normal and developmental morphology of the vesical ganglia in children.

A brief incursion was made into congenital defects and urinary tract infections to see whether either of the two had any bearing upon the morphology of these structures.

Regional distribution of vesical ganglia: Although the total number of ganglia seen in each section were counted, the values are given in terms of the number of ganglion cells contained therein. This is needed because a report of the number of ganglia would be meaningless as ganglia are of variable size and may contain from one to one thousand cells.

We utilized the sampling method outlined by Leibowitz and Bodian (1963). These authors commented and we concur on the difficulties of accurately estimating the number of ganglion cells present in the bladder wall. The larger ganglia are generally densely populated with cells and many are at different stages of maturation. This is particularly so in the young child. In spite of those factors there is general agreement between the values obtained in this study and those reported by Leibowitz and Bodian, although their data were based exclusively on sagital sections.

Direction of the blocks: It is generally recognized that random biopsies of the bladder seldom show ganglion cells. It was therefore of interest to determine whether the site or direction of the blocks has any
bearing on the number of ganglia that are visible or in their appearance.

We found no appreciable difference in the number of ganglia seen in sections from the longitudinal or the sagittal blocks when comparable areas of the bladder were considered. By either method it was consistently shown that the maximum concentration of ganglia is around the base of the bladder, particularly in the lateral aspect. This results from the distribution of the vesical plexus branches upon the wall of the bladder. It indicates the necessity, previously pointed out by Leibowitz, that biopsies for studies on the vesical ganglia be taken from those sites where the concentration is known to be greater and not random.

Symmetry in vesical ganglia: Although Leibowitz (1963) meticulously counted the number of ganglion cells in one hemibladder, their study did not permit conclusions in regard to symmetry or lack of it between the two sides of the bladder.

In this investigation the two halves of the normal bladder were compared and showed no apparent differences in distribution or morphology of the ganglia.

When the same procedure was carried out on an abnormal bladder, lacking ureter and kidney on one side, findings between the two sides were strikingly different. The ganglia were large and contained many more and larger cells than those of the normal side. Over 80 percent of the neurons were in advanced stages of degeneration.

In the normal side, only occasional cells within a ganglion showed some signs of degeneration. This was estimated in a careful survey of all sections to be not over 20 percent of the total. It could be of
additional interest to remark that the kidney on the unaffected side was mildly hypoplastic although of normal anatomical configuration.

The reason for the ganglion cells on the side of the renoureteric agenesis presenting quantitative and morphological differences with those from the normal side remain obscure. Autolysis can, however, be discounted as the bladder was fixed in distention with 10 percent formalin and then bisected. Both halves, therefore, should be equally fixed.

The differences in morphology seen between the right and the left half of the bladder may reflect a difference in functional activity. Marinesco (1909) stated that a nerve cell separated from the center from which it receives stimuli or from the center to which it sends functional impulses cannot live indefinitely without undergoing atrophy.

The concept of Marinesco could perhaps be applied to the vesical neurons which seemingly are degenerating on the side where the ureter and kidney are absent. But the vesical ganglia are not know to have any specific functional control of the motility of the ureter or of the uretero-vesical junction.

As there are no previous studies comparing the ganglia of the two halves of the bladder, additional information in asymmetry of the vesical ganglia is not available.

Pre- and postnatal development: The previous reports of Stohr (1928), Swenson (1953), and Leibowitz (1963), contributed valuable data in regard to the morphology, pathology, and distribution of these structures in the wall of the bladder.

Our study differs from theirs in having documented the pre- and postnatal development correlating chronologically the maturation of the
vesical and enteric ganglia in the same subjects. We know of no previous study in which these observations were made on the same subjects or on different subjects.

Coincidently with the onset of elimination of urine, agreed to occur between the fourth to the fifth month of fetal life (Smith, C., 1949), the ganglion cells of the bladder make rapid progress showing a strikingly advanced degree of maturation when compared with the enteric neurons of the same child or other children of the same age group. This finding was consistent in all cases. Most patients in this series were white, but in the few Negro infants studied no histological differences were noted.

As a tentative explanation it could be postulated that the ganglion cells of the bladder take part in the vesicorenal reflex (Langley, L. L., 1959; Narath, 1951), the pathway of which has not been yet demonstrated. Although not uniformly confirmed (Trueta, 1947), several well conducted investigations have shown that bladder distention inhibits urinary output by the kidney (Tolls, 1955). It is not known if the mechanism is reflex or humoral. If it was reflex, one could postulate further that the vesical ganglion cell from the ureterovesical junction perhaps send postganglionic fibers up the ureter to the kidney. Or if a humoral mechanism was considered instead, the presence of neurosecretory granules in autonomic neurons comes to mind. Since Scharrer (Lennette and Scharrer, 1946) demonstrated them in the sympathetic ganglia of the monkey, neurosecretory granules have attracted considerable attention. But their presence in neurons of the vesical ganglia has not been shown.
The appearance of the Nissl's substance in these neurons is peculiarly granulated and one cell may be markedly different from another in its tinctorial affinity, although normal otherwise. In the present state of knowledge, these color variations must still be interpreted as an incipient degree of degeneration. That it represents a functional state associated with a varying content of a neurohormone is strictly speculative.

The presence of a well defined nucleus, nucleolus, and sharply outlined nuclear membrane characterizes the mature neuron in our series. Cells that correspond to this description are seen in increasing numbers following birth and reach a peak between 6 and 10 years of age, although they are not as many as later in adult life. Therefore the assumption of de Castro (1932) that maturation takes place all through life is probably correct and applies to vesical neurons, as it does to enteric neurons (Smith, B., 1960).

Nature of the vesical ganglia: Sympathetic or parasympathetic?
A summary of the controversial data regarding the nature of the intramural vesical ganglia at level of the trigonus follows:

1. Pharmacological data indicate the ganglia present in the trigonus are all sympathetic (p. 13).

2. From denervation studies in the cat Moseley and Kuntz (1936) reported 50 percent of the ganglia as sympathetic and 50 percent parasympathetic. Studies in the dog by Iljina and Lawrentjew (1932) showed that vesical ganglia are exclusively parasympathetic.
3. Morphological data contributed by Stohr (1923) and BaKay (1938) in the human bladder indicate that the ganglia are sympathetic.

According to our observations the polarity noted in the vesical ganglia, particularly in those of the adventitia is a typical feature of the sympathetic ganglia (Plates 53-60). This characteristic is not seen in the ganglia of the enteric plexuses which are recognized as parasympathetic. Another characteristic that vesical ganglia, from the adventitia layer, have in common with the sympathetic ganglia is the thick, well organized capsule, quite different from that seen in the parasympathetic enteric plexuses. Although our study is strictly morphological we conclude that the large generally superficially located ganglia are probably sympathetic and those small, located deeper in the bladder wall are parasympathetic. Since innervation is likely to be different at the level of the trigonus than in the rest of the bladder, it is suggested that only a study in which the trigonus has been separated from the detrusor could contribute conclusive data in this point.

Vesical ganglia in congenital malformations: Four groups of patients (Table 7) presenting with malformations of the pelvic organs or of its innervation pathways were studied. Although none show obvious morphological abnormalities of the vesical ganglia, the number of representative cases is too small to permit definite conclusions. In addition the series does not include any patients with Hirschsprung's disease in which Swenson demonstrated a concomitant absence of colonic and vesical ganglia.
Effect of chronic urinary infection: It is of interest, however, that in 18 patients (Table 8) with chronic urinary infection, some having similar anatomical defects as those in Table 7, marked vesical ganglia abnormalities were seen. The biopsies had been taken from the lateral aspect of the bladder in most cases and ganglionic structures were present in all. Two thirds of the sections studied were from freshly fixed surgical biopsies obtained without resorting to electrocoagulation. The changes observed were not limited to the ganglion cells but involved the whole ganglionic structure and affected all the ganglia present (Plates 45-52). This was associated with an evident increase in number and caliber of nerve fibers. This association may recall the histological changes of the colonic plexuses in congenital megacolon (Hirschsprung's disease) but in this later entity the ganglionic structures are clearly outlined with no inflammatory changes present, ganglion cells absent and nerve fibers abundant.

Since all surgical biopsies were in patients over one year of age, the discrepancy between the size of the ganglia present and the expected normal for the age was marked.
SUMMARY

This study on the intramural vesical ganglia is based upon histological observations of bladder sections obtained by surgical biopsy and at autopsy in eighty-five human subjects, ranging in age from the third month of intrauterine life to the age of fourteen years. The following conclusions were derived.

Location: The study confirms that in the human, intramural vesical ganglia are located in large numbers around the base of the bladder and the uretero-vesical junction. Most large ganglia are located superficially in the adventitia, but many ganglia are present deep in the bladder musculature, submucosa and immediately subjacent to the mucosa.

Symmetry: Distribution was found to be similar in both halves of the bladder when the cysto-reno-ureteric anatomy is normal. In one case with absence of the renoureteric complex on one side, morphological differences in the vesical ganglia were noted when both sides of the bladder were compared.

Developmental progression of intramural vesical ganglia: Documentation was from the second trimester of intrauterine life to the 14th year of postnatal life. The changes were compared with those observed in the ganglia of the intestinal plexuses in the same subjects. Parallelism in development ceases at the 4th month when vesical ganglia show a quick increase in maturation characteristics. Presumptive explanations for such phenomenon are discussed.
Effect of congenital malformations of the pelvic organs or its neuroanatomical pathways: Of the malformations studied none seemed to influence the morphology of the vesical ganglia as long as urinary infection had not been present.

Effect of chronic urinary infection: When infection had existed, marked morphological changes were observed in the ganglionic structures.

We believe this to be the first study documenting the status of the vesical ganglia in the presence of chronic urinary tract infection.
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Smith, Blanca, 1960. Pre and Postnatal development of the plexuses of Meissner and Auerbach, Thesis (M. M. Sc.), Ohio State University.


Stewart, C., 1899. On the course of impulses to and from the cat's bladder, Amer. J. Physiol., 2: 182.


APPENDIX A
Table 1
Cases of the Prenatal Period, Second Trimester of Gestation

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Gestation age in Lunar Months</th>
<th>Weight in Grams</th>
<th>Crown-rump Length in Millimeters</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>4</td>
<td>3-4</td>
<td>28</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>4-3</td>
<td>200</td>
<td>130</td>
</tr>
<tr>
<td>6</td>
<td>4-5</td>
<td>250</td>
<td>140</td>
</tr>
<tr>
<td>7</td>
<td>5-6</td>
<td>480</td>
<td>300</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>650</td>
<td>320</td>
</tr>
</tbody>
</table>
Table 2

Cases of the Prenatal Period, Third Trimester of Gestation*

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Gestation Age in Lunar Months</th>
<th>Weight in Grams</th>
<th>Crown-rump Length in Centimeters</th>
<th>Lived</th>
<th>Autopsy Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>6-7</td>
<td>720</td>
<td>32</td>
<td>18 hs.</td>
<td>Pulmonary Atelectasis</td>
</tr>
<tr>
<td>10</td>
<td>7-8</td>
<td>1100</td>
<td>35</td>
<td>3 hs.</td>
<td>Prematurity</td>
</tr>
<tr>
<td>11</td>
<td>7-8</td>
<td>1150</td>
<td>38</td>
<td>3 days</td>
<td>Bronchopneumonia</td>
</tr>
<tr>
<td>12</td>
<td>8</td>
<td>1240</td>
<td>39</td>
<td>1 day</td>
<td>Pulmonary hyaline membrane</td>
</tr>
<tr>
<td>13</td>
<td>8</td>
<td>1280</td>
<td>39</td>
<td>1 day</td>
<td>Subdural hematoma</td>
</tr>
<tr>
<td>14</td>
<td>8-9</td>
<td>1520</td>
<td>41</td>
<td>2 days</td>
<td>Pulmonary hyaline membrane</td>
</tr>
<tr>
<td>15</td>
<td>8-9</td>
<td>1750</td>
<td>43</td>
<td>1 day</td>
<td>Pulmonary hyaline membrane</td>
</tr>
<tr>
<td>16</td>
<td>8-9</td>
<td>1880</td>
<td>45</td>
<td>2 days</td>
<td>Pulmonary hyaline membrane</td>
</tr>
<tr>
<td>17</td>
<td>9</td>
<td>1900</td>
<td>45</td>
<td>1 day</td>
<td>Pulmonary hyaline membrane</td>
</tr>
</tbody>
</table>

*All were prematurely born.
Table 3

Cases of the Neonatal Period
(Birth to 1 month)

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Gestation Age in Lunar Months</th>
<th>Weight in Grams</th>
<th>Crown-rump Length in Centimeters</th>
<th>Lived</th>
<th>Autopsy Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>Full term</td>
<td>3080</td>
<td>49</td>
<td>Still-born</td>
<td>Asphyxia at delivery</td>
</tr>
<tr>
<td>19</td>
<td>Full term</td>
<td>2950</td>
<td>51</td>
<td>6 hs.</td>
<td>Lacerated tentorium</td>
</tr>
<tr>
<td>20</td>
<td>Full term</td>
<td>2520</td>
<td>43</td>
<td>18 hs.</td>
<td>Pulmonary hyaline membrane</td>
</tr>
<tr>
<td>21</td>
<td>Full term</td>
<td>2840</td>
<td>64</td>
<td>1 day</td>
<td>Fibrocystic disease</td>
</tr>
<tr>
<td>22</td>
<td>Full term</td>
<td>2950</td>
<td>55</td>
<td>24 hs.</td>
<td>Complete transposition of great vessels</td>
</tr>
<tr>
<td>23</td>
<td>Full term</td>
<td>4100</td>
<td>52</td>
<td>1 day</td>
<td>Lacerated tentorium</td>
</tr>
<tr>
<td>24</td>
<td>Full term</td>
<td>2760</td>
<td>43</td>
<td>1 1/2 hs.</td>
<td>Aspiration</td>
</tr>
<tr>
<td>25</td>
<td>Full term</td>
<td>2890</td>
<td>35</td>
<td>3 days</td>
<td>A. V. communis</td>
</tr>
<tr>
<td>26</td>
<td>Full term</td>
<td>3230</td>
<td>49</td>
<td>4 days</td>
<td>Cortilocular</td>
</tr>
</tbody>
</table>
Table 4
Cases of the Infancy Period
(one month to two years)

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age at Death</th>
<th>Autopsy Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>3 months</td>
<td>Tetralogy of Fallot</td>
</tr>
<tr>
<td>28</td>
<td>3 months</td>
<td>Subarachnoid Hemorrhage, traumatic</td>
</tr>
<tr>
<td>29</td>
<td>4 1/2 months</td>
<td>Congenital heart defects, multiple</td>
</tr>
<tr>
<td>30</td>
<td>7 months</td>
<td>Multiple injuries, shock</td>
</tr>
<tr>
<td>31</td>
<td>7 months</td>
<td>Lacerated liver</td>
</tr>
<tr>
<td>32</td>
<td>2 years</td>
<td>Lacerated liver</td>
</tr>
<tr>
<td>33</td>
<td>2 years</td>
<td>Ruptured spleen</td>
</tr>
<tr>
<td>34</td>
<td>2 years</td>
<td>Ventricular septal defect</td>
</tr>
</tbody>
</table>
Table 5
Cases of the Early Childhood Period
(2 to 6 years)

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age at Death</th>
<th>Autopsy Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>2 1/2 years</td>
<td>Multiple injuries</td>
</tr>
<tr>
<td>36</td>
<td>3 years</td>
<td>Brain laceration</td>
</tr>
<tr>
<td>37</td>
<td>3 years</td>
<td>Lacerated liver</td>
</tr>
<tr>
<td>38</td>
<td>4 years</td>
<td>Ruptured spleen</td>
</tr>
<tr>
<td>39</td>
<td>5 years</td>
<td>Cervical cord transsection</td>
</tr>
<tr>
<td>40</td>
<td>6 years</td>
<td>Brain laceration</td>
</tr>
<tr>
<td>41</td>
<td>6 years</td>
<td>Gun shot wound of head</td>
</tr>
<tr>
<td>42</td>
<td>6 years</td>
<td>Head injury</td>
</tr>
</tbody>
</table>
Table 6
Cases of the Late Childhood Period
(6 to 14 years)

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age at Death</th>
<th>Autopsy Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>43</td>
<td>7 years</td>
<td>Massive head trauma</td>
</tr>
<tr>
<td>44</td>
<td>8 years</td>
<td>Lacerated brain</td>
</tr>
<tr>
<td>45</td>
<td>9 years</td>
<td>Compound skull fracture</td>
</tr>
<tr>
<td>46</td>
<td>10 years</td>
<td>Cerebral laceration</td>
</tr>
<tr>
<td>47</td>
<td>11 years</td>
<td>Head injury</td>
</tr>
<tr>
<td>48</td>
<td>13 years</td>
<td>Lacerated liver</td>
</tr>
<tr>
<td>49</td>
<td>14 years</td>
<td>Brain hemorrhage</td>
</tr>
<tr>
<td>Type of Anomaly</td>
<td>Case No.</td>
<td>Age at Death</td>
</tr>
<tr>
<td>--------------------------</td>
<td>----------</td>
<td>--------------</td>
</tr>
<tr>
<td>Urinary tract malformations</td>
<td>50</td>
<td>5 hrs.</td>
</tr>
<tr>
<td></td>
<td>51</td>
<td>6 hrs.</td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>12 hrs.</td>
</tr>
<tr>
<td></td>
<td>53</td>
<td>1 day</td>
</tr>
<tr>
<td></td>
<td>54</td>
<td>1 week</td>
</tr>
<tr>
<td>Abdominal wall deficiency</td>
<td>55</td>
<td>8 days</td>
</tr>
<tr>
<td></td>
<td>56</td>
<td>12 days</td>
</tr>
<tr>
<td>Anorectal malformations</td>
<td>57</td>
<td>6 hrs.</td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>4 days</td>
</tr>
<tr>
<td></td>
<td>59</td>
<td>6 days</td>
</tr>
<tr>
<td>Neuroaxial malformations</td>
<td>60</td>
<td>9 days</td>
</tr>
<tr>
<td></td>
<td>61</td>
<td>10 days</td>
</tr>
<tr>
<td></td>
<td>62</td>
<td>20 days</td>
</tr>
<tr>
<td></td>
<td>63</td>
<td>55 days</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>2 mons.</td>
</tr>
<tr>
<td></td>
<td>65</td>
<td>3 yrs.</td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>3 yrs.</td>
</tr>
<tr>
<td></td>
<td>67</td>
<td>4 yrs.</td>
</tr>
</tbody>
</table>
Table 8
Cases of Chronic Urinary Tract Infection

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age at Death</th>
<th>Autopsy Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>68</td>
<td>4 days</td>
<td>Posterior urethral valves</td>
</tr>
<tr>
<td>69</td>
<td>3 mons.</td>
<td>Posterior urethral valves**</td>
</tr>
<tr>
<td>70</td>
<td>4 mons.</td>
<td>Posterior urethral valves**</td>
</tr>
<tr>
<td>71</td>
<td>16 mons.</td>
<td>Polycystic kidney**</td>
</tr>
<tr>
<td>72</td>
<td>2 yrs.</td>
<td>Chronic pyelonephritis, bilateral</td>
</tr>
<tr>
<td>73</td>
<td>2 1/2 yrs.</td>
<td>Bladder neck obstruction**</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age at Biopsy</th>
<th>Clinical Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>74*</td>
<td>20 mons.</td>
<td>Bladder neck obstruction**</td>
</tr>
<tr>
<td>75*</td>
<td>1 yr.</td>
<td>Exstrophy of the bladder</td>
</tr>
<tr>
<td>76*</td>
<td>2 yrs.</td>
<td>Exstrophy of bladder</td>
</tr>
<tr>
<td>77*</td>
<td>25 mons.</td>
<td>Atresia of vesical neck</td>
</tr>
<tr>
<td>78*</td>
<td>4 yrs.</td>
<td>Bladder neck obstruction**</td>
</tr>
<tr>
<td>79*</td>
<td>2 1/2 yrs.</td>
<td>Bladder neck obstruction</td>
</tr>
<tr>
<td>80*</td>
<td>5 yrs.</td>
<td>Bladder diverticulum</td>
</tr>
<tr>
<td>81*</td>
<td>7 yrs.</td>
<td>Bladder diverticulum</td>
</tr>
<tr>
<td>82*</td>
<td>7 yrs.</td>
<td>Chronic cystitis</td>
</tr>
<tr>
<td>83*</td>
<td>10 yrs.</td>
<td>Bladder neck obstruction</td>
</tr>
<tr>
<td>84*</td>
<td>16 yrs.</td>
<td>Chronic cystitis—ureteritis**</td>
</tr>
<tr>
<td>85*</td>
<td>11 yrs.</td>
<td>Atonic bladder</td>
</tr>
</tbody>
</table>

*Bladder biopsies in cases No. 74 to No. 85 were obtained at operation.

**All had suprapubic cystotomies.
Plate 1. Ganglion in the superficial aspect of the muscular layer. Kluver-Barrera (X520)

Plate 2. Segment of a large ganglion containing many thousands of ganglion cells. Location: between muscular and adventitia. Kluver-Barrera (X100)
Plate 3. Two small ganglia (G) in between muscle bundles, deep in the bladder wall. Kluver-Barrera (X 50)

Plate 4. Ganglion (G) with approximately 10 ganglion cells located near the mucosa (a). Nerve endings extend between groups of muscle fibers (b) and (c). Kluver-Barrera (X 50)
Plate 5. In sections from the right half of the bladder, side of the renoureteric agenesis, ganglion cells are considerably larger and more numerous than on sections taken from the normal half (Plate 6 below). H. E. (X 100)
Plate 7. Bizarre forms are frequent among the large number of degenerated cells present in sections from the side of renoureteric agenesis. Several cells with double nucleoli are seen. H. E. (X 100)

Plate 8. This shows the maximum concentration of bizarre forms in the center of a ganglion, although cells in the periphery are also involved to a lesser degree. The ganglion is deep in the bladder musculature. H. E. (X 50)
Plates 9 and 10. Many cells from the anatomically abnormal bladder half showed, paradoxically, a more advanced degree of maturatiopn for the age than those from the normal side. Degenerative changes were affecting however most of the cells as is shown at magnifications of 1300X and 2700X, respectively. H. E.
Plates 11 and 12. The degenerative changes were more advanced in some areas where only occasional intensively basophilic shrunken neurons remained. H. E. (X 100)
MATURATION OF THE VESICAL GANGLIA, PRENATAL PERIOD: 2nd TRIMESTER

Plate 13. Loose stroma of bladder wall typical of fetal bladder. (Early second trimester) H. E. (X 650)

Plate 14. Cluster of early neuroblasts. Ganglion is not yet well defined but the future oval contour is already detectable. H.E. (X 650)
Plates 15 and 16. Illustrate small ganglia in between muscle bundles. H. E. (X 50)
Plate 17. Already during the second trimester peripherally located ganglia are larger and contain more cells per ganglion than those located deeper in the bladder wall. This remains a characteristic of human vesical ganglia. H. E. (X 50). Compare with Plates 15 and 16.
Plate 18. Section from intestine in Case No. 3, early 4th month. A continuous layer of neuroblasts is seen in the myenteric position; a few isolated groups in the submucous layer. Gallocyanin (X 50)

Plate 19. Bladder section, also from Case No. 3, illustrates the similarity in ganglion cell size between bladder and bowel. This feature persists until the 4th month. Gallocyanin (X 50)
Plates 20 and 21. Sections from fetal bladders in the 4th month of gestation showing progress toward capsule formation around each ganglion. Polarity of ganglia not yet evident. Neurons still in the early neuroblast stage. Gallocyanin (X 1000)
Plate 22. Case No. 5 (late 4th month). Marked increase in Nissl substance, more in some cells than in others. Nucleus still very dark and homogeneous. H. E. (X 650)

Plate 23. Sixth month fetus. Progression in ganglion cell size and maturity characteristics is evident although many early neuroblasts, still undifferentiated, are seen. The architecture of the capsule is better defined than previously. H. E. (X 650)
MATURATION OF THE VESICAL GANGLIA, PRENATAL PERIOD: 3rd TRIMESTER

Plate 24. 8 month fetus. Maturation also progressing on deeper ganglia but is not yet as advanced as in peripheral ganglia. In some cells the nuclear membrane is now sharply outlined. Gallocyanin (X 100)
Plates 25 and 26. Polarity of ganglia was first observed at the end of gestation. Generally when polarity is seen, a well defined capsule is already present. These sections from a nine month fetus show a ganglion from the adventitia layer with framework of nerve fibers. Kluver-Barrera. Magnification (X100) and (X520), respectively.
Plates 27 and 28. Both these fields demonstrate groups of nerve fibers of the type appearing at the end of gestation. They are more frequently seen in sections from the infancy and childhood periods. Their connection with ganglionar groups is not clear. H. E. (X 650)
MATURATION OF THE VESICAL GANGLIA, POSTNATAL PERIOD (FIRST MONTH)

Plate 29. Bladder section of a one month old infant. In the ganglion cell (G) the cytoplasm is faintly outlined but the nucleus and nucleoli are sharply defined. Nuclei of satellite cells (S) are abundant. Gallocyanin (X 1000)

Plate 30. In the non silver stained preparation the polarity of the ganglia is suggested by the disposition of the satellite cell nuclei. Gallocyanin (X 520)
Plate 31. Gradual progression in the complexity of the dendritic apparatus in deep intramuscular ganglia. Monopolar and bipolar neurons still present, however. Kluver-Barrera-Holmes (X100)

Plate 32. Similar status than on Plate 31. Section from vesicoprostatic junction. Bodian-Kluver-Barrera (X100)
POSTNATAL PERIOD (2 TO 6 YEARS)

Plate 33. Occasional multipolar neurons are seen but not of full size as yet. Bodian-Kluver-Barrera (X 650)

Plate 34. Neurons are now mostly multipolar but are still increasing in size. The few small neurons remaining in the ganglia presumably progress to adult size gradually. Bodian-Kluver-Barrera (X 650)
Plate 35. Bladder section from a 2 year old child. Polarity in ganglia is more and more frequently observed in large ganglia comet shaped, even though buried in the muscular layer. Kluver-Barrera (X100)

Plate 36. In the mature vesical ganglia neurons are large, peripherally located and of multifaceted contour. A capsule is generally well demonstrated. H. E. (X 520)
Plate 37. Nerve trunks and large groups of ganglion cells with a cell population of many thousands (G) are now common in the adventitia. H. E. (X100)

Plate 38. Individual cells in those large ganglia have well defined cytoplasm, nucleus and nucleoli. Kluver-Barrera (X520)
Plate 39. Two multipolar neurons of complicated dendritic apparatus. Bodian-Kluver-Barrera (X650)

Plate 40. Although the multipolar state has definitely been reached, neurons at different stages of development are still seen as in this large ganglia. Kluver-Barrera (X100)
Plate 41. A group of vesical ganglion cells from a newborn with posterior ureteral valves. Ganglionar capsule is still poorly defined. Nuclear membrane is sharp but two nucleoli are present in most of the cells illustrated. H. E. (X520)

Plate 42. A section from the bladder of an infant born with deficient abdominal wall musculature shows a ganglionar group located in the adventitia and nerve fibers between the bladder musculature. Size of neuron normal for age. Excentric nucleolus. H. E. (X520)
Plate 43. Six day old. Diagnosis: Rectal Atresia. Partial view of a small intramuscular ganglion with well defined capsule. One neuron and portion of another. H. E. (X520)

Plate 44. Bladder section from a three year old child with dorsolumbar mylomeningocele shows a ganglion with fully mature cells exhibiting mild degenerative changes. H. E. (X520)
THE EFFECTS OF INFECTION UPON THE VESICAL GANGLIA

Plate 45. Ganglion showing degenerative changes in the great majority of the neurons present, which are also considerably smaller than average for the age of the patient (2-1/2 yrs.). Gallocyanin (X520)

Plate 46. View of the same field at X1000 shows the disorganization of the ganglionar structure, the invasion by satellite cells and the predominance of bizarre forms among the ganglion cells remaining.
Plates 47 and 48. Fields from surgical biopsies of the bladder of children over six years of age with chronic urinary infection. The nuclei of many satellite cells and occasional "ghosts" of ganglion cells (gh) together with abundant nerve fibers are seen. No intact ganglion cell remains. Gallocyanin (X520)
Plate 49. Although ganglia are poorly outlined, nerve fibers are abundant often in the proximity of submucosa and mucosa. In addition, nerve fibers seem thicker than normal. Gallocyanin (X520)

Plate 50. Only a few ganglia with typical oval contour remain but well preserved ganglion cells within may be almost entirely absent as in this field. Note reduced size of neurons in spite of magnification (X520). Compare with Plates 33 to 40 for the normal appearance of vesical ganglia in children after 2 years of age. This patient was 7 years old.
Plates 51 and 52. Fragment of a ganglion with well preserved capsule but replacement of most neurons by satellite cells. The few ganglion cells remaining are well below the average size and configuration expected for the age of ten years. H. E. (X520)
TYPICAL FEATURES OF MATURE VESICAL GANGLIA

Plate 53. A well defined capsule of variable thickness is present on all mature vesical ganglia, even in those located deep between muscle bundles and having just a couple of neurons. Kluver-Barrera (X100)

Plate 54. The capsule is thicker in ganglia situated in the adventitia. The elongated ganglion "A" to "A" containing thousands of ganglion cells has features suggestive of sympathetic origin. Kluver-Barrera (X50)
Plate 55. A close up of the capsule (C) suggests it is composed of several cell layers. Nuclei of capsular cells are well demonstrated. H. E. (X1000)

Plate 56. Blood vessels are seen frequently adjacent to those ganglia located in the adventitia. Gallocyanin (X50)
Plates 57 and 58. Capsule and polarity are demonstrated in a ganglion located in the submucosa layer. Relationship to blood vessels is also observed in this location. Kluver-Barrera (X50) and (X520), respectively.
Plates 59 and 60. These views of vesical ganglia deep in the bladder wall best illustrate the typical appearance of the vesical ganglia in its mature stage. Child 10 years. H. E. (X520) and (X1000)