THE BACTERIOLOGY AND PATHOLOGY OF

STERILITY IN CATTLE

A Thesis

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# INTRODUCTION

Sterility may be defined as the inability to produce young.[1] It may occur in either sex, but the most disasterous effects are produced when the female is no longer capable of reproducing her kind.

## Importance of Sterility to Agriculture

The question of continued propagation of the species in man is a sociologic one, while in the domesticated animals the economic considerations far exceed others in importance. Agriculture is a basic industry and animal husbandry bears to agriculture an inseparable relationship. The human race is concerned especially in the production of beef and milk and since the toll from sterility is greatest in cattle the economic factor of the disease must be of interest to all mankind.

Oftentimes the development of a race or family of cattle is dependent upon the successful rearing of calves from the very cows temporarily or permanently rendered sterile. The valuable pure-bred animal's worth is intimately related to her ability to produce calves of equally high value. When no longer capable of reproduction her breeding cannot be used as a source of revenue and she must be sold for beef. In the grade herd the importance of the disease to the owner lies in loss of milk production, when likewise he must dispose of a high producing cow for beef prices. A cow

may be regarded as a machine, and must produce from the fuel she consumes, salable goods in the form of milk and calves; if this is no longer possible her value is small, depending upon market quotations.

In the male the loss from sterility is comparable, but occurs less frequently and therefore is of less concern.

We may thus sum up: Sterility is one of the most important diseases of cattle. It strikes at the very heart of the animal industry, rendering at times losses which are irreparable.

## Historical Resume

Sterility is not new, for mention has been made of it in veterinary and medical literature since the very beginnings of these branches of science. Until the brilliant researches of Lister and Pasteur led the way to modern and rational conception of disease no advances were made in solving the problem. It must be admitted that although bacteriology, pathology and surgery have contributed much to our present knowledge, the physiologist has also added knowledge to the science which is invaluable in dealing with the problems of sterility.

Toward the end of the nineteenth century the interest of scientific men was attracted to the field of comparative and veterinary pathology and bacteriology, and much study was devoted to the diseases of the genital tract of animals. At this period the incidence of the disease seems also to have increased, probably because of changed conditions in the development of our civilization, and to the increased production of livestock. Infectious abortion in cattle also became more widespread and coincident with it

sterility in cows increased.   The inference is not to be drawn
that infectious abortion sprang up suddenly, but rather that it
became a disease of increased importance due to the advances in
production of livestock.   It is true also that it appeared to be
more prevalent because of better recognition by an increased number
of scientific observers.   It is not the purpose of this thesis to
discuss infectious abortion of cattle.   This subject, since the
time of Bang (1897)[2], has received the attention of scores of
competent workers.[3]   The fundamental facts of infectious abortion
are well described.   Since infectious abortion is so closely related
to the increased prevalence of sterility a passing mention should
be made.

The fact that animals may abort is conveyed to us in Genesis,
Chapt. 31, verse 38.   In 1907 "Complete Farmer" mentions abortion
as being contagious and so regarded by farmers.[4]   In 1885 Nocard's
investigations[5] quite clearly set forth the contagious nature of the
disease, although he did not recognize the specific infection.
Woodhead, Aitken, M'Fadyean and Campbell in 1889[6] showed that
abortion could be produced in cows by inserting into the vagina
plugs of wool, contaminated by the discharges of aborting cows.
Until 1897, however, the etiology still remained in doubt.   Through
the remarkable work of Bang[2] in Denmark, the specific etiological
agent was described.   This is regarded without serious question as
the cause of the clinical entity, "infectious abortion."   The work of
Bang was confirmed in England in the "Report of the Departmental
Committee to Inquire into Epizootic Abortion", 1909.[7]   In America
the disease was first definitely established by the identification
of the organism B. abortus (Bang), by MacNeal and Kerr in 1910.[8]

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Other early workers who deserve mention for their contributions to the knowledge of infectious abortion are: Zandel, Styrl, Franck, Roloff, Brawer and Johne. Although not meeting with ultimate success their studies did much in keeping the subject alive and open to further researches.

In the writings of veterinary obstetritians we find the first mention of diseases leading to sterility in animals. The first veterinary work on this topic appeared in Belgium by Eberhard and Günther (1793).[9] This was followed by the text of Skellet[10] which made its appearance in England (1807). From this period on, publications appeared in practically every country, but to the English speaking people no real information was collected into a text until Fleming[11] in 1877 wrote his "Text Book of Veterinary Obstetrics." Hill (1882)[12] in his text, "Bovine Medicine and Surgery", devotes a short chapter to sterility in the male and female. The noted veterinarian, Dieckerhoff, in his "Lehrbuch der Speciellen Pathologie und Therapie fur Thierarzte" (1888), gives excellent pathological descriptions of diseases involving the genitalia of the cow. For instance, he classified metritis as acute, peracute, septic and chronic catarrhal endometritis. Cook[14] and Awde[15] reported independently, cases of bovine endometritis in 1891. In the short paper by Awde we read, "Although I have been in practice for 15 years (1876) I cannot recollect that I ever saw a case of this kind prior to the first one here recorded (1889).

The real beginning of our knowledge concerning diseases of the genitalia of the cow dates from the time of Bang, followed by such contributers as Wall, Holth, Nielsen-Sorring, Albrechtsen, Hess, Tuff, M'Fadyean, Zwick, Jensen, MacNeal, Theobald Smith, Schroeder

and Williams.    Many others could be mentioned but these were pioneers in their respective countries.

To summarize:  The modern conception of sterility has developed during the past twenty-five years, through advances in physiology, bacteriology, pathology and therapeutics.  The fundamental sciences have placed the veterinary gynecologist in a position to more successfully cope with the diseases of the genitalia.

## Development of the Genital Tract of the Cow

Before describing the results of the present investigation, a review of the development of the organs of the genital tract will be given.  This, together with their anatomy and physiology, to be described later, is necessary for a clear understanding of pathologic changes that may occur and lead to sterility.

## Development of the Uterine Tubes, Uterus, and Vagina

Early in embryonic life, before the genital ridges have become well marked (about the 2nd mo.) the ducts of Müller (Müllerian ducts or tubes) develop as two ridges near the peritoneum, near to but independent of the Wolffian ducts.  They appear at first as solid rods, and later develop into definite tubes, ending blindly for a time, then opening into the cloaca.  When "hollowed out" each tube opens into the peritoneal cavity at the future fimbria of the uterine tube.  Fusion later takes place caudally, for a variable distance toward the anterior, thus forming a single tube of the fused portion.  The latter is to become the vagina, vestibulum, cervix uteri and corpus uteri.  The anterior ends become the uterine tubes and the middle portion the cornua uteri.  A good description

of the development as occurring in the bovine species is given by
Wall[16], as follows:

"The uterus, uterine tubes and vagina are developed from a
double mesodermal tube, the Müllerian tubes. These tubes caudally
merge, forming the corpus and cervix uteri, and the vagina. During
the fetal period the vagina opens first into the cloaca (formed by
the entoderm), and then into the sinus urogenitalis (formed by
division of the cloaca membrane), then adjoins the ectoderm. The
epithelium of all three layers of the blastoderm, the simple
columnar epithelium of the entoderm and mesoderm and the early
stratified epithelium (stratified pavement epithelium) of the
ectoderm then meet in the sinus urogenitalis (the future vestibulum).
Early, even in the third month in the embryo of cattle (two invest-
igations of embryos of 9.5 cm.) the stratified epithelium of the
ectoderm seems, however, to cover the wall of the vestibulum (and of
the urethra). The simple columnar epithelium of the mesoderm,
bordering on the pavement epithelium of the ectoderm, which at the
limit is at first double, then stratified, with a rather sharp
limit, which is visible first after great microscopic enlargement.
This continues until the fifth month (three examinations of embryos
of respectively 18, 24 and 27 cm.), after which a slow inward grow-
ing of the ectoderm epithelium into the vagina is observed replacing
it. A transformation of the mesoderm epithelium into the stratified
epithelium seems not to be going on. The microscopic examinations
evidently favor the hypothesis of an inward growth, as the stratified
epithelium often has a staining reaction, differing from the meso-
derm epithelium, but agreeing with the ectoderm epithelium
(especially visible with the Unna-Pappenheim method), and further, as

in the first place the connection by itself, and in the second the
gradual transition from a high stratified epithelium to a double
epithelium indicate a derivation from the ectoderm epithelium.  This
"ectodermoisering" proceeds at different rates of speed in different
individuals (five examinations of embryos of respectively 4, 4-1/2,
4-1/2, 8 and 9 months old).   In new-born and other animals (ten
examinations:  two new-born calves, two of the animals were calves
about 2 months old; two were heifers about 2 years old, and four
older cows) the limit of the ectoderm epithelium was, as a rule,
observed at the os uteri externum, or in young animals one to
several centimeters behind this, but of the four older cows the
stratified epithelium in two cases was observed in the cervix and in
one of these cases up to 4 cm. in front of the os uteri externum.
It seems, therefore, as if with years the stratified epithelium
spreads somewhat at the expense of the columnar epithelium, a fact
which is, according to Sobotta[17], already known concerning woman.
Several accouchements are considered to favor this phenomenon, which
is clearly a consequence of a greater regenerative capacity of the
stratified epithelium.

"In the uterus mesenchyme the differentiation between mucosa and
muscular coat first makes its appearance at the end of the fourth
month and the primitive carunculae begin to be visible at the same
time as swelling of the mucous membrane.  First in the ninth month
are the uterine glands observed, first as solid then as tubular
epithelium formations, growing into the mucous membrane, and the
new-born calf shows a distinct layer of glands along the entire
uterus, except in the carunculae."

## Development of the Ovary

At a very early stage of the mesonephros, a narrow strip of mesothelium extending along the medial surface of this structure becomes thicker and the cells become arranged in several layers. These cells become differentiated into two kinds: (1) Small cuboidal cells, with cytoplasm staining rather intensely and (2) larger spherical cells with clearer cytoplasm and larger vesicular nuclei. The latter are the sex cells, and the whole epithelial (mesothelial) band is known as the germinal epithelium. The sex cells are destined to give rise to the sexual elements, in the female to the ova, in the male to the spermatozoa. This differentiation cannot be made at first, but appears at a later period.

The cells of the germinal epithelium increase in number by mitotic division and for some time at least, the sex cells continue to increase in number by differentiation from the small cuboidal (indifferent) cells, as indicated by the presence of intermediate stages between the two types. Their germinal epithelium soon becomes spearated into two layers, (a) a superficial layer which retains its epithelial character and contains sex cells, and (2) a deeper layer composed of smaller cells which resemble those of the mesenchyme, and which give rise to a part, at least, of the stroma of the sex glands. The elevation formed by these two layers projects into the body cavity from the medial side of the meso-nephros and constitutes the genital ridge. From the superficial epithelial layer columns or cords of cells, containing some of the sex cells, grow into the underlying tissue. About the end of the first month of pregnancy changes begin in the genital ridge, which differ according to whether ovaries or testicles are to be formed.

The ovarian characters are: (1) The surface epithelium does not become flattened; (2) a layer of surface epithelium, corresponding to the tunica albuginea of the testicle, grows between the epithelium and the deeper parts. This layer, however, is of a looser nature; (3) there is a sharp line of demarcation between the cell columns and the stroma; (4) the sex cells continue to increase in size and become more conspicuous.

During the processes of development, the anlage of the genital gland becomes more or less separated from the mesonephros and finally is attached only by a thin sheet of tissue - the mesovarium. At the same time it begins to grow more rapidly in thickness becoming oval in shape.

The loose connective tissue spoken of above as corresponding to the tunica albuginea of the testicle, grows in between the surface epithelium and the cell columns (sex cords) and effects a more or less complete separation. The sex cords are thus pushed farther from the surface and become marked off from the surrounding stroma and constitute the so-called medullary cords. The cortex of the ovary at this stage is represented only by the surface epithelium, which is composed of several layers of cells and contains numerous sex cells in various stages of differentiation. The ovary may thus be said to be composed of two parts - (1) the rete anlage and (2) the stratum germinativum. The latter is subdivided into (a) medulla and (b) cortex.

1. The <u>rete cords</u> develop into a group of anastomosing trabeculae which constitute the rete ovarii, situated in the hilum but nearer the cephalic end of the gland. They are the homologues of the rete testis. The cells composing them are smaller and darker than those

of the medullary cords. In some of the cords lumina appear and are
lined with irregular epithelium. This stage represents the height
of their development in the ovary and from this time on they atrophy,
and gradually disappear.

2. (a) The medullary cords are composed of small epithelial
cells and a large number of sex cells. These are surrounded by a
stroma. During foetal life they give rise to primary ovarian
follicles, later they degenerate and finally disappear.

(b) The cortex of the ovary, at first consists of several
layers of small, darkly staining cells, among which are many large,
clearer sex cells, or primitive ova. From the epithelium, masses or
cords of cells grow into the underlying tissue, carrying with them
some of the primitive ova. These masses are known as Pflüger's
egg cords. The epithelial cells give rise to the follicular cells
and are seen constantly undergoing mitotic division. The primitive
ova increase in size and their nuclei show a chromatic network.

The egg cords break up and become separated from the surface
epithelium so that a single ovum is surrounded by a single layer of
epithelial cells. These constitute the primary Graafian follicles.
The formation of egg cords is usually completed at birth but in rare
instances continues for a short time after birth. The stroma
increases so that the egg cords become separated by a considerable
amount of connective tissue. Their germinal epithelium becomes
reduced to a single layer of epithelial cells.

Each primary ovarian follicle, containing a primitive ovum, is
composed of a single layer of flat or cuboidal cells, plus a layer
of stroma, which gives rise to the theca folliculi. After the
follicular epithelium has become several layers thick, a fluid

substance known as the liquor folliculi, and probably derived from these cells, is secreted around these cells. As the follicle enlarges these pools coalesce and form a single large pool, lying in the center of the follicle. Thus the epithelial cells are crowded toward the outside, where they form a layer several cells in thickness, known as the stratum granulosum. The ova is likewise crowded off to the periphery of the follicle. The little elevation of the stratum granulosum in which the ovum is imbedded is known as the cumulus oöphorus. As the follicles approach maturity they gradually enlarge, pushing through the cortex until the tunica albuginea is reached. Various views are held in regard to the size that the follicle may acquire. In woman the size averages 5 mm. but varies[18]. In.the cow Albrechtsen[19] states that "a follicle is regarded as mature when it reaches a diameter of 10-15 mm. According to Hess[20], 10-14 mm.

It must be borne in mind that development of the follicles is slow and they do not reach maturity until the age of puberty. In heifers this may be the 5th, 6th, 7th, or 9th month, but according to Fleming[21] the age of puberty is from 12-18 months. Staint Cyr[21], gives the age of puberty in the bovine species at from 12 to 14 months. De Bruin[22] states that on the average the cow reaches puberty at ten months of age. When the follicle reaches maturity and other conditions being favorable, it ruptures at the surface of the ovary, and the ovum is set free.

It has been estimated that from 10,000 to 36,000 primitive ova appear in each human ovary, a vast number of which, it may be seen, never reach maturity. The number of ova in the ovary of the cow at birth, varies considerably. Heitz[23] estimated the number to be 3800,

while according to Käppeli's[24] investigations the number varies

within wide limits, (6740 to 297,668).

## Anatomy of the Genital Tract of the Cow

### 1. The Bones of the Pelvis

The pelvic girdle (Cingulum extremitas pelvinae) consists of the

ossa coxarum, which unite ventrally in the symphysis pelvis, and

articulate with the sacrum dorsally.

The os coxae is the largest of the flat bones. It is made up

of three parts, the ileum, the ischium, and the pubis, which meet to

form the acetabulum, a large cotyloid cavity for articulation with

the head of the femur. These parts are fused at about 1 year of

age.

The pelvic inlet is eliptical and oblique. In a cow of medium

size the conjugate diameter is 23 to 24 cm., and the transverse

diameter about 18 cm.

The ligaments of the pelvic girdle constitute important

structures in the support of the generative organs:   These are

(1) dorsal sacro-iliac ligament, a strong band which is attached to

the tuber sacrale and the summits of the sacral spines;  (2) lateral

sacro-iliac ligament, is a triangular thick sheet which is attached

in front to the tuber sacrale, and adjacent part of the median

border of the ilium, above the great sciatic notch, and below to the

lateral border of the sacrum;  (3) sacro-sciatic ligament, is an

extensive quadrilateral sheet, which completes the lateral pelvic

wall.  Its dorsal border is attached to the border of the sacrum and

the transverse processes of the first and second coccygeal

vertebrae.  Its ventral border is attached to the superior ischiatic

spine and tuber ischii. Between these it bridges over the lateral border of the ischium and completes the lesser sciatic foramen. The anterior border is concave and completes the greater sciatic foramen.

## 2. Vagina

The vagina is the passage which extends nearly horizontally, through the pelvic cavity, from the neck of the uterus to the vulva. In the non-pregnant animal its length is 20-25 cm. In pregnancy its length increases to 30 cm. or more. The retro-peritoneal pouch of peritoneum extends backward about 12 cm., on the dorsal surface; ventrally the serous coat extends backward but 5 cm. In the ventral wall of the vagina, between the muscular and mucous coats there are commonly present two remnants of the Wolffian duct, the canals of Gartner. When well developed (or occasionally cystic) they may attain the diameter of 3 mm., and may be traced forward to the anterior portion of the vagina or even farther along the broad ligaments toward the ovary. They have an opening posteriorly toward the external urethral orifice. Röder states, according to Sisson[25], that the right canal was absent in 52 percent of cases, the left in only 22 percent.

The vulva has thick, wrinkled labia, and both commissures are acute; the ventral one is pointed, and has on it a number of long hairs. It lies about 5 cm. behind, and about the same distance below the ischial arch. The external urethral orifice is 10 to 12 cm. from the ventral commissure. Beneath the opening there is a blind pouch; the suburethral diverticulum, which is about 3 cm. long and into which the finger may easily be admitted. The two glandulae vestibulares majores (glands of Bartholin), are situated in the

lateral walls of the vulva, under the constrictor vulvae. They are about 3 cm. long and about 1.5 cm. in width. Each has two or three ducts opening into a small pouch of the mucous membrane; this opens just above the floor of the vulva about 3 to 4 cm. lateral to and behind the external urethral orifice. The gland is lobulated being separated into lobules by connective tissue and muscular bands. The glandulae vestibulares minores occur along the median ventral groove. Numerous lymph nodes are present in the mucosa and may be large enough to cause slight prominences. The clitoris has a very short crura, but the body is 10-12 cm. long, and is flexuous, only the pointed end of the gland can be seen in the ventral commissure of the vulva.

The wall of the vagina is composed from within outward of a mucous membrane, a muscular and a fibrous coat. The epithelium is stratified ectoderm epithelium, which in older animals always extends forward covering the os uteri externum, where transition occurs to the columnar epithelium as seen in the uterus. The mucous membrane has no mucous glands, the mucous seen in it supposedly being derived from the cervix and uterus. The muscular coat is composed of fibers having a longitudinal direction. The muscle is unstriped. The fibrous coat is dense and thick and is composed in part of areolar tissue. The peritoneum is reflected over it in places, forming a serous coat.

The vagina is richly supplied with blood vessels and nerves. The arteries are derived from the branches of the internal iliac, viz.: the vaginal, internal pudic, and uterine. The veins correspond. There is a close network of lymphatics throughout the mucous membrane, these draining toward the internal iliac nodes. The nerves

are derived from the sympathetic, through the pelvic plexus.

### 3. Uterus

The uterus lies almost entirely within the abdominal cavity in
the adult. It is a uterus bicornis, the body being relatively
short. The cornua are relatively long. As a rule the body is under
4 cm. in length but may occasionally be that long. The muscular
and fibrous coats of the inner portions of the cornua walls are
connected for a considerable distance anterior to the body, and are
invested by a single peritoneal investment. For this reason upon
external examination the cornua appear to be 15-18 cm. in length,
while in reality the length is 35 cm. Their breadth at the body is
about 3 cm. The cornua taper gradually toward the uterine tubes
where the size is gradually carried out without an abrupt transition
from uterus to tube. The free part of the horn curves at first
downward, forward and outward, and then turns backward and upward,
forming a spiral coil. In the non-gravid organ the mucous surfaces
of the uterine walls are normally in contact with each other.
Posteriorly the cavity of the uterus is continuous with the vagina,
through the cervical canal.

The cervical canal (canalis cervicis) is a tortuous channel
about 7 cm. or more in length. According to Sisson[25] the length
is 10 cm. It can easily be seen that it is dependent for its
length upon the length of the cervix uteri.

The cervix uteri consists of a constricted portion of the uterus,
the circular muscle fibers being more numerous than in the uterus.
The walls are very dense, (muscular and fibrous) and measure about
3 cm. in thickness. The lumen (cervical canal) in the bovine

species is spiral-shaped. Because of tight closure it is difficult to dilate. The walls on either side are in quite close apposition except in estrum and during and following parturition. Posteriorly a portion of the cervix projects into the vagina for a distance of 3-5 cm., in the form of a cone, the diameter of which varies greatly but according to Williams[26] approximates its longitudinal dimensions. The projection into the vagina is properly called portio vaginalis uteri, or os uteri externum. The tip presents posteriorly many longitudinal converging folds of its mucous membrane which reminds one of a radiating flower. The cervix anteriorly joins the corpus uteri and is called at that point the os uteri internum.

Microscopically the uterine wall is seen to be made up of three coats: (1) mucosa (the endometrium), (2) muscularis (the myometrium) and (3) serosa (the perimetrium). There is no sub-mucosa. In thickness the mucosa ranges from 1.5 mm. to 4 mm. The surface is lined by high columnar epithelium which in the cornua is 30-50 microns in thickness, and in the body about 20 microns. The mucosa of the bovine uterus, is especially elaborated in parts for the accommodation of the fetus. To these parts the name carunculae, or maternal cotyledons is applied. It is this specialized formation, especially in the bovine uterus, which forms the maternal placenta of pregnancy and in which connections between the fetus and mother develop. Thus we may divide the mucosa into two parts: (1) The carunculae, (2) the gland mucosa.

1. Carunculae. These are oval prominences, about 100 in number either irregularly scattered over the surface or arranged in rows. They are covered by the ordinary uterine columnar epithelium, and in the virgin uterus form little swellings over the surface of the

mucosa. They are 3 mm. in diameter and 1.5 in depth. Beneath the
epithelium are found no glands but numerous connective tissue cells
(old and young fibers) and a few scattered lymphocytes throughout
the connective tissue reticulum. Numerous large and small vessels
are also noted not a great distance from the epithelial surface.
The carunculae do not reach the muscular coat, but only approximate
it. There are usually in the area above the muscular coat a few
uterine glands, which open at the sides of the carunculae, never
upon their surface. In pregnancy the carunculae undergo a remark-
able evolution, seemingly an embryological development, similar to
that seen in the uterine glands. Wall[27] considers them as depots of
embryonal tissues of great vegetative power. He states that they
appear as epithelium and connective tissue with vessels, which
develop in pregnancy and undergo involution after parturition. "In
pregnancy these carunculae attain the weight of 200 gr., with
dimensions up to 9 cm. (length)by 7 cm. (breadth), by 6 cm. (thick-
ness), with a stem about 6 cm. long, and 3 cm. broad growing thinner
at the caruncula." They present a convex inner surface and are on
stalks instead of being sessile. The carunculae in the pregnant
uterus show per sq. cm. about 10 crypts, often as many centimeters
deep, resembling in form a three, four or five-edged, obtuse pyramid
narrowing irregularly toward the peritoneum (outside). Inwards,
(nearest the embryo) they are 0.3 mm. but at the bottom only about
0.1 mm. in width.[27] The surface has a deep sponge appearance due to
this development. The walls of the crypts are composed of closely
arranged fibers and rather numerous fibroblasts, and a few
scattered lymphocytes and polymorphonuclear leukocytes. The
epithelium of the crypts is simple, low cuboidal, with rather

numerous emigrating leukocytes. At the bottom there is a dense, highly vascular connective tissue. Into the carunculae crypts the choronic villi of pregnancy grow, one growing into each crypt and branching like a tree to fill the various nitches.

After pregnancy has ended the carunculae undergo involution, corresponding to the involution of the entire organ. The reduction in size is rapid, so much so that within three days after parturition they are only one-tenth the volume of those of the last month of pregnancy. Hemorrhages and hyaline degeneration can be observed in the carunculae stromata, together with necrobiosis of the cells.[27] The necrobiosis begins at the lumen of the uterus and goes outward (toward the peritoneum). Occasionally vessels may show white thrombi. In the crypts the pavement epithelium shows defects. Cells are observed, among which are red blood corpuscles, lymphocytes, polymorphonuclear leukocytes, and remains of choronic villi which show a granular degeneration. The next step in the involution begins about the fifth day after parturition and ends between the tenth and fourteenth, during which time the dropping off of the carunculae occurs, as described by Hilty, and Sommer.[28] It may thus be seen that the loss of the carunculae with considerable mucous membrane, corresponds to the sloughing of the decidua in deciduate animals (as in the primates). The whole caruncula rarely sloughs off, and when so, only in small portions. The necrotic material passing away in the lochial fluid. After the birth of one calf the carunculae remain larger than in the virgin uterus, reducing in volume to 10 mm. in length (diameter) and 4 to 5 mm. in thickness. They are often slightly depressed at the center. They show in this state a loosely reticular connective tissue, supporting

the normal high uterine epithelium of columnar type, through which may be seen fibroblasts, solitary lymphocytes, and in its deeper portion there are many large vessels. These have thick walls and fine lumina, some showing hyaline degeneration of the intima and thickened media.

2. The gland mucosa. This portion has as its principle difference from the carunculae the presence of numerous uterine glands. It consists of two layers, (a) an inner layer, called by Ellenberger[31] stratum cellulare. This is about 0.5 mm. in thickness. In this area one finds numerous fibroblasts, scattered lymphocytes, a few polymorphonuclear leukocytes and a finely arranged network of connective tissue through which numerous capillaries may be found. (b) The outer layer is thicker, contains a greater number of connective tissue fibers, fewer wandering and lymphoid cells, but has in connection with it simple convoluted tubular uterine glands. No sub-mucosa exists. The simple columnar epithelium of the uterine glands is without cilia, which generally holds true for the surface epithelium of the mucosa. In gravid and involuted uteri, however, there may be cilliated processes projecting into the lumen, which Wall[28] regards as being only the fibrinous product of secretion. The number of tubuli shown in the uterus at different stages of the gravid and non-gravid uterus Wall's[28] figures are here quoted, "The maiden uterus in two-month or older heifers, calculated on four uteri, shows 600 to 700 cross-cut tubuli per centimeter of mucous membrane in sections of 10 microns. These tubuli have an average diameter of 17 microns. The gravid uterus (three uteri) shows an average number of 100 to 150 tubuli

with a diameter of about 150 microns. Within three days post
partum there can be counted 100 to 200 tubuli with a diameter of
50 microns. The involuted uterus (six uteri) 300 to 1000, on an
average about 700 tubuli - with a diameter of about 17 microns."
It may thus be seen that the tubuli grow very large during
pregnancy, but regain more or less of their former size in the
involuted uterus.

The muscular coat is composed of an inner circular layer of
unstriated muscle, and an outer longitudinal layer, beneath the
serosa. These fibers are much enlarged in the gravid state of the
uterus. The fibers interlace closely, and are disposed in bundles
or layers, and are intermixed with areolar tissue, containing a large
number of blood vessels, lymphatics and nerves. The areolar tissue
is more abundant near the outer surface.

The external layer of the muscular coat lies directly beneath
the serosa;  a large number of muscle bundles begin as longitudinal
bands in the cervix and are continued through the cornua and
eventually pass off into the uterine tubes.  The inner muscular
layer and the point of ramification of the blood vessels before
they pass into the mucosa serves to mark off the mucous membrane
from the muscular coat proper. The width of the muscular coat is
from 1.5 to 6mm.

The serosa is a special investment  of the peritoneum, consist-
ing of a layer of flattened endothelial cells, supported by a thin
elastic membrane. It shows but few alterations in the evolution
and involution of the uterus.

In structure the cervix is similar to that of the uterus. The
mucosa is free of glands, (in this respect differing from the human

cervix), and is much plaited by longitudinal folds, so that in
cross-section it appears as a mural crown. The mucosa of the
cervical canal is also thrown into transverse circular rugae, three
to four such folds being present. The epithelium is for the most
part single and low columnar, except that of the os uteri externum
(portio vaginalis uteri) which is covered by a stratified ectodermal
epithelium, as seen in the vagina. "In old cows this may spread
forward 4 cm. in front of the os uteri externum by "ectodermoiser-
ing." Although no cervical glands exist, its epithelium, through
the presence of goblet cells, secretes a mucous, having an important
role to play in the formation of the seal in pregnancy. The fold-
ing of the epithelium adds much surface for mucus secretion. The
muscular coat is very dense and thick, the circular fibers being
much more abundant than in the body or horns of the uterus. The
serosa, where existing, is similar to that seen at other portions,
previously described.

The arteries of the uterus and cervix are derived from the
uterine and the uterine branch of the utero-ovarian, and the
internal pudic. Veins accompany the arteries. Lymphatics are
numerous and drain toward the internal iliac and lumbar lymph
nodes. The nerves are derived from the pelvic and uterine plexuses.

## 4. Uterine Tubes

The ovaries are regarded as glands for the production of ova,
and the uterine tubes (tuba uterina) represent their ducts. They
do not immediately start at the ovaries, but open up into the
peritoneal cavity near that organ where they present a small orifice

surrounded by their fimbria. The fimbria comes into close
relationship with the ovary and may be attached at one end of the
ovary. When the ova are discharged they fall upon the fimbria and
are transported thru the lumen of the uterine tube, to the uterus.
At the uterine end of the tube, its communication is reduced some-
what in size, forming the isthmus. Between the isthmus and
fimbriated extremity is found the ampulla (near the fimbriated end).

In the bovine, the tube is thin and tortuous and is supported by
a specialized peritoneal reflection. Its entire length is from
21 to 28 cm. At the isthmus the thickness varies from 0.8 to 1 mm.
and at the ampulla it is from 3 to 5 mm. in diameter.[29]

The uterine tube shows microscopically, three layers: The
tunica mucosa, tunica muscularis and the tunica serosa. The
mucosa is supported by a tunica propria or basal membrane and is
thrown up into folds, projecting for a greater or lesser distance
into the lumen of the tube. In the bovine these folds vary in
number from 20 to 40. The epithelium, which is supported by the
stroma of the tunica propria, is simple (or pseudostratified),
ciliated and high columnar in type. It covers the mucosa through-
out the length of the tube./is composed of both circular and
The tunica muscularis
longitudinal fibers of plain muscle. The circular layer is much
the stronger and is located nearer the lumen of the tube, the
longitudinal fibers are seen external to the circular and are less
developed. The muscularis is strongest near the uterine end of the
tube. The serosa is a specially reflected portion of the peritoneum
(serous endothelium) which covers the external portion of the tube,
forming its tunica serosa.

The arteries are derived from the utero-ovarian, and the veins
are satelites of the arteries. The lymphatic vessels pass with the

ovarian vessels to the lumbar lymph nodes. The nerves come from
the sympathetic, renal and aortic plexuses.

## 5. The Ovaries

The ovaries are two small, oval, somewhat solid looking bodies,
lying one on each side of the pelvis, and projecting into the
peritoneal cavity, at the posterior part of the broad ligament,
which is itself formed by a fold of peritoneum. During sexual
life a number of clear vesicles can be seen near the surface of the
ovary, sometimes even projecting beyond the surface. These are the
ripe or ripening Graafian follicles. There may also be seen
occasionally one or more yellow masses imbedded in the ovary,
the corpora lutea. These project slightly from the surface of the
ovary, in the form of a small rounded elevation. Their dimensions
on the average are 3½-4½ cm. in length, 2-1/3-2-2/3 cm. in breadth,
1-1/3-1-1/2 in thickness, with a weight of from 10 to 15 grams.
The right ovary is slightly larger than the left.[30]

Each ovary is formed of a solid mass of fibrous looking tissue,
which contains between its fibers very many elongated cells, like
those of embryonic fibrous tissue. The stroma is more condensed
near the surface where it forms the tunica albuginea. The ovary
projects into the peritoneal cavity, but is not actually covered by
a serous membrane. The peritoneum ceases abruptly at the attach-
ment to the broad ligament. Along this line of attachment (hilum),
blood vessels and nerves enter and leave the ovary. In this
location frequently are seen a large number of interstitial cells,
much like those seen in the testis. Similar cells may occur
scattered in groups about the stroma, but do not seem to be numerous

in the bovine ovary.   A layer of short, clear, columnar epithelial

cells (the germinal epithelium) covers the entire clear surface of

the ovary.   Primitive ova may occur among them.   Here and there,

especially in the young animal, the epithelium is thickened and

dips into the stroma of the ovary.   These have been described in the

embryology of the ovary as Pflügers egg tubes (or cords)   In the

development of the ovary these cords become broken up into islands

of epithelial cells, from which the Graafian follicles arise.

Imbedded in all parts of the cortical stroma, except near the hilum,

are a large number of spherical or ovoid vesicles, each containing

an ovum, together with other cells.   These vesicles are the Graafian

follicles.   They are of all sizes ranging from the size of the ovum,

and entirely microscopic to 15 mm. in diameter.   The smallest

Graafian follicles contain only a small ovum and a single layer of

epithelial cells surrounding it.   The largest contain an ovum of

mature size (about 0.2 mm. in diameter) surrounded by a considerable

mass of epithelial cells, known as the cumulus oöphorus, by which it

is attached to the wall of the follicle.   Layers of the same cells

line the wall continuously, and constitute the stratum granulosum.

The wall itself is fibrous and known as the theca folliculi.   The

large follicles are distended with a clear, straw-colored fluid, the

liquor folliculi.   As the fluid accumulates the follicles project

from the surface, as little convex, translucent elevations.   It is

at this point that the follicle eventually bursts, liberating the

ovum surrounded by its cumulus oöphorus.   The stratum granulosum

remains in situ and undergoes proliferation, developing into the

epithelial tissue of the corpus luteum.   The smallest Graafian

follicles (primary follicles) are found near the surface of the

ovary, in the so-called cortical layer. As the follicles develop
they sink into the stroma, and as they become enlarged through the
elaboration of their liquor folliculi (probably secreted by the
membrana granulosa) again approach the surface. Many follicles
never go beyond the primary follicle stage but after a time undergo
a degenerative change by either atrophy or atresia of the follicle.
The theca folliculi undergoes growth and organization, finally
replacing the follicle. These are known as atretic follicles.
The ova are large spherical cells with clear nuclei and large
nucleoli. They are enveloped by a clear membrane, known as the
zona pellucida. The ovum is composed of clear cytoplasm, the
vitellus, a large vesicular nucleus, the germinal vesicle, within
which is a single dark staining nucleolus, or germinal spot. The
cytoplasm (vitellus or yolk) contains many granules, especially near
the central part. The nucleus (germinal vesicle of Purkinje)
measures about 0.05 mm. in diameter. Following the rupture of the
Graafian follicle, and the escape of the ovum, there is considerable
hemorrhage. This blood clots in the former cavity of the follicle
and gives rise to the corpus rubrum. The follicular wall begins,
almost immediately, to hypertrophy and becomes thrown up into folds
or plaits. The greater part of the hypertrophy is from a thickening
and proliferation of the epithelial cells of the stratum granulosum,
followed by inward growth of vascular processes chiefly from the
theca folliculi. The corpus rubrum undergoes dissolution and
absorption before this advancing growth, which when completed is
known as the corpus luteum. The cells of the corpus luteum are
known as lutein cells, and grossly appear to be yellow. The pigment
is for the most part carotin with small amounts of xanthophil.[31]

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The corpus luteum is at first sharply marked off from the surrounding ovarian stroma, by the theca folliculi. After a time it becomes less sharply differentiated from the stroma. The lutein cells undergo absorption and connective tissue replacement, which later becomes contracted and subjected to hyaline changes. Thus in ovaries of aged animals white scars are prominent throughout the stroma, which often even cause an irregular scaring of the ovarian surface. These remains of the corpora lutea, are known as corpora albicantia or simple sclerotic corpora lutea.

The arteries are derived from the ovarian arteries which are large and flexuous and reach the ovary by passing between the layers of the mesovarium. Veins are large and numerous. Lymphatics drain toward the lumbar nodes. Nerves are derived from the sympathetic through the renal and aortic plexuses. The vessels enter the ovary at the hilum.

## 6. Attachments of the Genital Organs

The body and cornua of the uterus are attached to the abdominal and pelvic walls, by two extensive peritoneal folds, the broad ligaments (Ligamentum lata uteri). These extend on either side from the upper part of the flanks, about a hand's breadth below the level of the tuber coxae, to the dorsal border of the cornua and the lateral margins of the body of the uterus. They contain the blood vessels, lymphatics, and nerves of the uterus and ovaries, connective tissue, and a considerable amount of unstriped muscle which is continuous with the smooth muscle of the uterus. The ureters are situated along their parietal margins. The lateral layer of each gives off a fold, the round ligaments of the uterus, (ligamentum teres uteri) which blend with the parietal peritoneum, and can be

distinctly traced to the vicinity of the abdominal inguinal ring.
The anterior extremity of this ligament is situated above the
extremity of the cornu, and forms a long, round appendix.   It
contains muscle tissue, vessels and nerves, and is the homologue of
the gubernaculum testis.   The anterior part of the neck is
continuous with the vagina, and thus has a more fixed position than
the rest of the organ.

## The Development and Structure of the Fetal Membrane

Fertilization of the ovum usually occurs in the isthmus of the
uterine tube, after which it descends into the uterus, sometime
during the first week of pregnancy, still surrounded by its zona
pellucida.

Upon arrival of the fertilized ovum in the uterus, the con-
dition of that organ is typical of that of the unimpregnated organ
(to be described later).   The glands contain very little secretion
and no trace of blood or debris.   The stroma cells increase in
numbers and become more dense, leukocytes begin to invade the
epithelium (they are very numerous below the epithelium, in the
stroma).   The ovum soon becomes implanted in the uterine wall, and
the foetal membranes develop about it.   A destruction of the
uterine epithelium begins about this time (18 days) over the
carunculae, and extends to all parts of the uterus that are in
contact with the growing embryo.   Regeneration of the epithelium
over the carunculae does not occur until after parturition, while
over the intercotyledonary areas (the gland mucosa), regeneration
takes place about the third month of pregnancy.

About the fertilized ovum there develop, soon after conception, certain appendages, these are: (1) The chorion, with the placenta foetalis, (2) the amnion, and (3) the allantois. These serve to protect and carry maternal nourishment to the fetus.

"The chorion (or vascular membrane) is the outer envelope surrounding the foetus and the two inner membranes, and adapts itself closely to the outer surface of the mucous membrane of the uterus." [22] Its development permits it to extend into the non-impregnated horn and corpus uteri. On its external surface there are tufts of villi which constitute the fetal, cotyledonous placenta. The chorion arises partly from the superficial layer of the morula (ectoderm) and partly from the embryonic parietal meso-derm, which develops later. The trophectoderm forms the outer layer of the blastodermic vesicle, which internally is made up of a mass of cells. The trophectoderm forms the primitive ectodermal layer of the chorion, which is composed of a single layer of cuboidal epithelial cells, and several ovoid binucleated cells. The former probably correspond to Langhans' layer of the human chorion, the latter to the syncytium. These represent the growing elements of the chorion, the choronic villi. The mesoderm gives rise to a connective tissue stroma supporting the ectodermal layer, which also accompanies the trophoblast of the villus, forming a connective tissue core or supporting stroma. The villi grow into the swollen carunculae and this union constitutes the fetal and maternal placenta. The exchange of nutritive material takes place between the fetal placenta and the mother through absorption and imbibition of maternal juices. There is no direct communication between the maternal and fetal circulations. The chorionic villi

approximate the large vessels of the carunculae and obtain for the
fetus materials essential to its life. The fetal blood vessels
follow the stroma of the chorion and thus ( as they arise from the
allantois) the blood borne waste products of fetal metabolism are
also eliminated and taken up by the maternal uterine circulation.
In ruminants the chorion is united to the amnion and allantois over
the middle of its inner surface, by loose gelatinous connective
tissue. On the surface of the cotyledons of the cow is found a
fluid, in the nature of an emulsion (uterine milk). It is a whitish
or reddish emulsion of alkaline or neutral reaction, with a
specific gravity of 1.036. Gamgee[22] found in 1000 parts of
uterine milk: Water 879.1 parts, solids 120.9 parts, the latter
being represented by albumin (with the cells) 104 parts; alkaline
albuminates, 1.6 parts; fats, 12.33 parts; organic salts, 3.74
parts; also some creatin, creatinin and xanthin. Colin[22] regarded
it as a post mortem product of decomposition, while other
experimenters regard it as possessing an important nutritive
function.

The amnion begins to develop as soon as segmentation has been
completed and the ovum has reached the uterus. It appears at
approximately the end of the second week of pregnancy. The develop-
ment of the amnion has been traced to the external layer of the
blastoderm. It begins at the umbilicus and surrounds the fetus like
a sac, enclosing it entirely, except at the point of origin, where
it takes part in the formation of the umbilicus. It represents a
thin, double membrane of little resistance. In ruminants the inner
surface is studded with little yellow projecting points, called by
Claude Bernard[22], "plaques glycogenique", for they are composed of

glandular tissue capable of producing glycogen. In this respect it is held that this function replaces the function of the fetal liver, until that organ is developed, (Joulin, St. Cyr, and Violet in "Traité d' Obsteterique").[22] Lecoq[22] holds that they are simply changed parts of the fetal skin. They are most numerous about the amniotic portion of the umbilicus. The external surface of the amnion is partly in contact with the allantois latterally and at the back of the fetus with the face of the chorion. The inner of the two membranes, previously mentioned, constitutes the true amnion, while the external one forms the external or false amnion, which by fusion replaces the prochorion thus forming the primitive chorion. Later when the allantois grows out it blends with it to form the permanent or allantoic chorion. This outer or false amnion is merely a portion of the external wall of the blastodermic vessicle, without having had its relations changed exteriorly. The space between the amnion and fetus constitutes the amniotic cavity and is distended with a clear straw-colored fluid. It is oval in shape with a depression at the umbilicus, thus giving to it a kidney or bean shape. As it becomes filled with fluid and enlarges it invests the vitteline stalk and allantoic cord, in its passage through the cavity. In the later part of pregnancy the fluid becomes mucoid, opalescent and stringy, and may contain fetal feces (meconium), giving it a brownish tinge. The amount of amniotic fluid at birth averages up to 4 kg. It serves a mechanical function protecting against injury and to lubricate the vagina during parturition.

The allantois arises as an invagination from the hind gut, just posterior to the vitelline duct, and grows outward and backward between the two amniotic layers (or if considered as by some authors

between the chorion and amnion). It is a continuation of the
urachus and is sacular in shape from which it receives its name,
allantois, (Gr. sausage). The urachus forms a tube for
communication between the allantois and urinary bladder of the
fetus. The external face of the allantois rests on the internal
face of the chorion. When the allantois is isolated and emptied
it is seen by inflation to be a bicornual, irregular, cylindrical
sac, the head of the fetus lying in the shortest cornu, the
posterior extremities in the larger. The allantoic sac contains the
allantoic fluid (the fetal urine), which gradually accumulates. It
is a whitish foamy, thin fluid of neutral reaction, which later in
pregnancy becomes yellow or brownish. Near the end of pregnancy it
reaches 8 to 15¼ L. in amount. Robin[22] has proven the absence of
glucose, oxalates, albumin, mucus and allantoidin in the allantoic
fluid. The allantois is important in transporting the fetal blood
vessels to the chorion, and to the chorionic villi of the placenta,
where the transference of maternal nourishment to the fetus occurs.
It also acts as a reservoir of fetal urine.

Other important appendages are the vitteline or yolk stalk,
supplying nutrition to the developing embryo until maternal
connections can be obtained. It is not of importance beyond this.
It arises from the primitive gut. The umbilical cord is composed
of urachus, two umbilical arteries, two umbilical veins and the
remains of the yolk stalk. All of these are imbedded in a
gelatinous mass, "Wharton's Gelatine" or Jelly. The sheath of the
umbilical cord is formed from folds or invaginations of the amnion.
The urachus carries the fetal urine to the allantois. The umbilical
arteries supply the fetal membranes and as stated previous through

their circulating blood, exchange fetal katabolic products for
maternal nutritive products, in the placenta.

## Physiology of Reproduction

### The Estrus Cycle

The cycle of events leading to estrum (or heat) is known as the
diestrous cycle. The cycle is usually divided into four stages:
(1) proestrum (period of growth and congestion, followed by the
period of destruction), (2) estrum (beginning period of recuperation-
"heat"), (3) metestrum (period of recuperation), (4) diestrum
(period of rest). If conception does not occur at any estrus period
during the sexual season, a prolonged period of rest may follow.
This is known as the anestrus period. The latter is ordinarily
shown only by certain species of animals having definite breeding
seasons. The complete cycle of events, including anestrum, is
known as the anestrous cycle.

The period of proestrum and estrum are commonly referred to as
the "heat period." As a matter of fact there is a marked
physiological difference in these two periods, proestrum correspond-
ing to menstruation in the human female, in which there is an
increased activity of the uterine glands, an increase in capillaries,
resulting in congestion of the uterus. Frequently at this time
bloody or clear mucus containing polymorphonuclear leukocytes and
other white blood cells can be seen coming from the glands, followed
by epithelial destruction and bleeding . The amount of blood is
slight in the cow, but is occasionally seen in the vagina, when
looked for. In the sheep, where careful studies have been made,

bleeding is a well established fact. This corresponds to the menstruation of the human female but is much less marked. The period lasts 2-4 days, as a rule. The proestrum is a process preparing the uterus for implantation of the ovum in its wall and corresponding as it does to menstruation does not occur as Williams states after estrum, but before as was pointed out by Haape[32] and later by Marshall.[33] Any other conclusion would make necessary the changing of our entire conception of the physiology of proestrum or menstruation.

Proestrum is followed by _estrum_ which is of very short duration. It marks the climax of the estrous cycle. At this time the female is willing to take the male and fruitful coition rendered possible. The uterus is in the beginning of recuperation, the glands are less active, hyperemia subsides and healing has commenced. Large numbers of leukocytes can be seen in the stroma. Some are phagocytic for the cellular detritis and blood pigment remaining from the proestrus.

If conception occurs this period is followed by the _period of gestation,_ which in the bovine has an average duration of 275 days.

On the other hand should conception not occur during estrus, the latter is succeeded by a short _metestrum,_ during which activity of the generative organs subsides and they gradually return to normal.

In some animals the metestrum may be succeeded by a long period of quiescence, the _anestrum_, while in others it is followed simply by a short period of rest; this interval is known as the _diestrum._ Following this period of rest, proestrum (period of growth and congestion followed by period of destruction) again begins, and the diestrous cycle is complete. It is probable that the uterine

changes do not take place abruptly but that each day marks certain differences in the progress of the cycle. The latter has been well studied in the human female and in the apes, where a menstruation similar to that in woman occurs. In woman the cycle may be divided into four stages:

(1) The Constructive Stage.

(2) The Destructive Stage.

(3) The Stage of Repair.

(4) The Stage of Quiescence.

The completion of the diestrous cycle according to Ellenberger[65] varies from two to four weeks. Wallace[59] states that estrus occurs among cattle, in the summer, every nineteen days, but in winter every twenty-first or twenty-second day. After parturition the cow usually appears to be in heat again within four or five weeks. The domestic cow is polyestrous, having several recurrent breeding periods at more or less regular intervals each year.

## The Ovarian Factor in Reproduction

Through continued experimental evidence it has been adduced that the diestrous cycle is definitely controlled through the activity of the ovary. Heat and menstruation are brought about by the elaboration of some sort of chemical substance or hormone, having its source in the ovarian tissue. This so-called internal secretion, not only controls the functional activity of the uterus, but also has to do with the development of the mammary glands and other sexual characteristics of the female.

The earlier view of Pflüger that the functional correlation between ovary and uterus was through the nervous mechanism is no

longer tenable.[34] Neither is the occurrence of menstruation
connected with the ripening of the Graafian follicle, for Heape has
shown that in monkeys menstruation may occur in the absence of
either ripe Graafian follicles or corpora lutea.[32] It has also
been noted that in bats copulation occurs in the autumn, and
ovulation is not observed until the following spring, the
spermatozoa being stored in the uterus during the period of
hibernation. It can thus be seen that the Graafian follicle can
have little to do with ovulation. Fraenkel[35] has stated that
menstruation is brought about by the effect of an internal
secretion elaborated by the discharged follicle or corpus luteum.
He also regards the corpus luteum as the only ovarian organ of
internal secretion and that it is responsible for the estrus cycle
as well as the raised nutrition of the uterus during pregnancy.
Fraenkel regards as proof of the importance of the corpus luteum
as an organ of internal secretion, the fact of its reoccurrence
following the menstrual period in women, and that it regenerates
monthly. This, like the above theory, is held by Marshall[34] to be
untenable, because it will not account for the sexual phenomena in
the lower animals. Most animals ovulate when the proestrum is
over, so that the corpus luteum is usually not present when estrum
or "heat" begins. Further proof exists in the female rabbit and
cat where ovulation occurs only after copulation, and in the absence
of the male, corpora lutea are not formed in the ovaries, yet these
animals show periodic heat periods.

To summarize all these facts it seems as Marshall states, the
proof of "heat" cannot lie in the internal secretion elaborated by
the corpus luteum, but the theory does seem to explain the raised

nutrition of the uterus during gestation, and also is a determining factor in the development of the mammary glands and in the continued existence of the fetus in the uterus at least during the early period of pregnancy. Marshall[34] has shown that "heat" is not dependent upon the development of the Graafian follicle nor upon the corpus luteum and suggests that its occurrence must be accounted for by the secretion from the interstitial cells, although no proof can be adduced showing cyclic changes in these cells. Marshall states further that, "The ovary is an organ providing an internal secretion which is elaborated by the follicular epithelial cells or by the interstitial cells of the stroma. This secretion circulating in the blood induces menstruation and heat. After ovulation, which takes place during estrum, the corpus luteum is formed, and this organ provides a further secretion whose function is essential for the changes taking place during the attachment and development of the embryo in the first stages of pregnancy." It is probable also that it is intimately concerned, through its internal secretion, with the development of the mammary glands, and that the hormone elaborated by the ovarian stroma has to do with the maintenance of the general metabolic equilibrium, and the development of female secondary sexual characteristics. The role of the ovary in influencing the general metabolic state has long been known, and has an intimate relation with other glands of internal secretion, changes in one of which seem to derange the others. The possibility exists that this latter function resides in the corpus luteum but definite evidence of its function in the ovary is still unknown.

That corpora lutea do not play an important role in the estrus

cycle is shown too, by the fact that they are not present during proestrum, and as ovulation occurs in either late proestrum or estrum, they can be only functional subsequent to their appearance, that is, after ovulation. Fraenkel maintains that the corpus luteum of the previous ovulation induces estrum of the succeeding one, but as a matter of fact, the previous corpus luteum has generally degenerated into the albicans before the succeeding ovulation appears. Further proof lies in finding that in certain animals estrum and ovulation do not necessarily occur simultaneously. It is a well-known fact that in cows corpora lutea retained pathologically, cause cessation of estrum and of ovulation, and that the removal of the corpus luteum by manipulation per rectum, will within about 3 to 7 days cause the appearance of heat, and the animal may conceive if bred at this time.[36] It seems therefore that this is additional proof of Marshall's theory, that the ovarian secretion having control of estrum and ovulation must be sought for in other portions of ovarian tissue. The lutein tissue, it would seem, inhibits ovulation, which may be another function of that body during pregnancy where both ovulation and heat are inhibited. Pearl and Surface[37] have shown that extracts of corpus luteum when injected into laying hens inhibit ovulation, a further support of the inhibitory action theory of the lutein secretion. Inhibitation may be the result of mechanical blocking, but more likely the active secretion of the corpus luteum inhibits the ovarian function, (or the ovarian secretion) controlling estrum and ovulation, for, as previously stated, its removal when pathologically retained, causes estrum to appear. It is also possible that its secretion, if controlling estrum as Fraenkel states, is only elaborated in the period of its regression, a fact which would correspond to the

cyclic changes that actually appear. The lutein secretion being most abundant during the latter stages of the regression of the corpus luteum, or accumulative, would correspond to the phenomenon of menstruation, which in itself might be responsible for the phenomenon of heat appearing after the proestral or menstrual state. The climax reached, the uterus undergoes a period of rest and repair. During this period a new corpus luteum is formed from the ruptured follicle and as it regresses begins to secrete. Thus the cycle recommences. This is hypothetical and probably not what actually occurs for Marshall points out that heat appears regularly in rabbits in the absence of the lutein cells.

The entire consideration must be only theoretical until the active principles of the internal secretion can be isolated chemically and definitely separated. The fact remains, however, which is the important thing, that the ovary has internal secretions intimately connected with developmental and sexual changes.

## Classification of Sterility

No thoroughly workable classification of sterility of animals, from a pathological viewpoint, exists. From the clinical standpoint an animal is sterile when she no longer reproduces young, but the causes are so diversified and prognoses likewise so variable that a fundamental classification is difficult, when based upon etiology and pathology. Many authors regard sterility as a symptom of disease, or when no demonstrable reason can be adduced for the condition, as an entity in itself.

The basis for the first subdivision is sex; (1) due to the male, (2) due to the female. This report is confined exclusively to the

latter. A further subdivision of either (1) or (2) might be
adopted as follows: (a) <u>Congenital</u> (usually absolute), and result-
ing from absence or arrested development of certain organs of
reproduction. Congenital displacement, although rare, may also be
a contributing factor. (b) <u>Acquired</u> (relative or absolute), and
the result of (1) acquired displacements or injuries, (2) tumors or
cysts, (3) specific inflammations, (4) non-specific inflammatory
reactions.

## General Consideration of Sterility

Sterility may be said to result, as a rule, when there are
deviations from the normal in the genitalia. Factors which are
considered essential to conception are: (1) A normal ovum; (2)
healthy and active spermatozoa; (3) normal tubal, uterine and
vaginal secretions, (4) a healthy endometrium in which the fertiliz-
ed ovum may lodge and develop. Inability to produce young is
normal before puberty and in aged cows, also occasionally in heavy
lactating animals. Gebauer[38] suggests that cows be regarded as
sterile (1) when they have passed their second year without showing
signs of estrum; (2) in stables where sterility is prevalent cows
which show no signs of estrum for a period of 8 weeks; (3) when
after calving estrum has not appeared within two months.

## Congenital Defects of Organs of Reproduction

The complete absence of the ovaries is rarely met with, while
arrested development, although not common, is more frequently seen.
In bovine twins, sexual development is often arrested (90% of cases
according to Williams) when the twins are of opposite sex. These

heifers, for the incomplete development almost always occurs in the female, are known as freemartins. Such individuals are absolutely sterile, unless the defect is a minor one. As a rule the arrest in development leaves the animal without uterus or only a partially formed one, or without a normally developed vagina. These animals are unquestionably sterile. In other cases where only minor defects of the uterine tubes, uterus or ovaries exist, conception may occur. This arrest in development is sometimes seen in heifers, not of twins. We have studied two cases in this work, where arrest in the development of uterus and vagina existed. There were in place of the uterus only partially developed horns and fibrous cords, the remains of the Müllerian ducts. These will be described in detail later. Thus we may have complete absence of or arrested development of any essential organ of reproduction. Whether the animal will be sterile or not depends upon the extent of involvement.

## Diseases of the Ovaries

A. <u>Active</u> and <u>passive</u> <u>hyperemias</u> and <u>anemias</u> may occur but are not important from the standpoint of sterility. Active hyperemia occurs in estrum and in infections, the passive type when a general passive hyperemia exists. Anemias exist in a general anemic state, and due to obstruction of arterial flow.

B. <u>Cystic changes</u> are among the most frequently encountered diseases of the ovaries. Although prevalent cysts are not of as much importance in sterility as was first supposed. Zangger[39] in 1859 seems to have been the first to study this condition. Since, Hess, Albrechtsen, and Williams have contributed to our knowledge of

the condition in cattle.

Ovarian cysts may be divided pathologically as follows:

    1. Simple ovarian cysts (retention cysts)

        a. Originating from the Graffian follicle.

        b. Originating from the corpus luteum.

        c. Originating from the remains of the Wolffian

          ducts.

    2. Pseudomucinous cystadenomata.

    3. Serous cystadenomata.

Pathologists are not agreed on a pathological classification of
ovarian cysts.  Some hold that they all have a common origin from
tubular ingrowths of epithelium.  Most workers favor a division
into a. Retention cysts, b. cystic neoplasms.  Retention cysts may
be of three types: (1) Failure of the Graafian/to develop normally
or failure to rupture with subsequent failure of the corpus luteum
to form.  Thus the follicle becomes distended as a follicular cyst.
(2) Cystic degeneration of the corpus luteum, at any time during
its development, but usually when pathologically retained.  (3)
Another type of retention cyst is séen especially at the hilum or
on the broad ligament.  These form from cystic remains of the
Wolffian duct and attain a great size.  This type is infrequent in
cows.

Superficial follicular cysts cannot be distinguished grossly
from normal follicles except that they be larger than the ripe
follicle, but size alone is no criterion.  As often occurs they
are small and multiple.  Again they may be large and single.
Frequently too, ovaries containing one large cyst and two or three
smaller ones are noted.  These cysts vary in diameter from

microscopical size to 18 to 25 mm. (or more), the average being between these extremes. They are thin-walled when occurring superficially, but when in the deeper ovarian structures the walls are thicker. The follicular epithelium which is always in part retained in the follicular variety becomes stretched and flattened and lies on its basement membrane. The cavity contains a straw-colored serous fluid, usually thin but often mucinous with or without the presence of the ovum (usually without). Sometimes the Graafian follicle only partially develops and then begins to degenerate. The stratum granulosum may be partly destroyed. The cavity is always distended with fluid, which microscopically appears as a dense homogeneous eosin staining mass. Such cysts may be small and multiple or enlarge to a great size. These will be described in detail in the case reports.

Corpus luteum cysts are similar in appearance, except that in place of follicular epithelium around the inner wall, lutein cells and connective tissue are always found. The fluid is often darker in color and may even be blood stained. It is usually thin but in one of our cases, it was of a thick gelatinous consistency and yellow in color.

Embryonal remains of the wolffian ducts occasionally are cystic but are very infrequent in cattle. They are most often found on the broad ligament.

The cystadenomata are not often described in veterinary literature and are therefore of little importance in sterility. Kitt[40] reports them as developing to the size of 20-30 or even 90 kilos in weight in the mare and cow. They are true neoplasms (benign) arising from ingrowths of either Pflügers cords or superficial

ovarian epithelium. This ingrowth ordinarily is not continued
after birth but when it occurs it gives rise to these adenomatous
structures. Cavities lined by simple cuboidal epithelium supported
by a connective tissue membrane is the characteristic picture
presented. The growth of epithelium spreads from the parent cyst.
Daughter cysts are thus produced until a multilocular arrangement
is formed. The epithelium secretes either a serous fluid or a
pseudomucinous one, quite jelly-like in consistency. Either simple
or papillary arrangement of the epithelial lining may be the typical
appearance. When completely removed they do not reoccur, neither do
they metastasize, but grow from the original focus, along the
abdominal peritoneum until eventually they may approximate the
border of the liver.

The etiology of retention cysts is not known. Rosenau and
Davis[41] have associated Streptococcus viridans with cystic disease
of women. Fitch[42] in his studies found streptococci and B. coli
in cystic ovaries. Now, 1921, he states he regards his early find-
ings as accidental. Loeb[43] has been able to produce cysts by
underfeeding guinea pigs. Only by excessively underfeeding was he
able to produce the condition, and then with considerable regularity.
Ewing[60] states that the "Fairly constant occurrence of inflammation
of the pelvic organs points to the inflammatory origin of these
cysts." It is possible that abnormal conditions or infections of
the uterus exert a reflex influence upon ovarian function with
possibly cysts as one of the ovarian changes produced. In regard to
etiology of cystodenomata we know nothing more than that they are
neoplasms, arising probably as has been suggested.

The symptoms produced by cystic ovaries are usually those of

nymphomania. Frequent and irregular heat periods with usually inability of conception are often observed, because of abnormal ova or absence of ovulation. Absence or suppression of estrum is sometimes also seen as a sequel to cystic ovaries.

C. Pathological retention of the corpus luteum is often noted. It has no special pathology, simply a retention and hypertrophy of the "yellow body", which for some unknown reason fails to degenerate into the corpus albicans. Its retention usually results in suppression of ovulation and estrum. If estrum occurs copulation is rarely followed by conception. Usually, after some weeks the corpus luteum becomes encapsulated within a firm connective tissue wall. The lining cells are connective tissue, within which are lutein cells, the remains of the corpus luteum. The etiology is unknown.

D. Sterility may result from a general deranged metabolic state through the influence it may have upon the ovary. Glands of internal secretion have been demonstrated as playing an important part in the physiological development of the female, and in general body health. Over-work, insufficient food, high protein diet, obesity and emaciation all render the ovary less responsive and sometimes result in temporary sterility or weakened and irregular estrum.

E. Oöphoritis. Inflammations of the ovary are acute or chronic. The acute types are most often associated with abscess formation from extention of peritonitis, perimetritis, or salpingitis. The chronic types produce proliferative changes with resulting scars and adhesions. The causes may be tuberculosis, actinomycosis, or pyogenic infections produced by streptococci, staphylococci or

<u>Bacillus</u> <u>pyogenes</u>. The infection usually enters the ovary follow-
ing rupture of a Graafian follicle. Sterility produced thereby is
usually absolute.

F. <u>Tumors</u>. Both carcinomata and sarcomata are rarely primary
in the ovary, the former being the most common. Dermoid cysts and
teratomata are also not infrequently seen, but in cattle are also
rare. The presence of such growths result in loss of ovarian
function with or without symptoms of nymphomania, and result in
sterility.

G. <u>Senile</u> <u>atrophy</u> <u>of</u> <u>the</u> <u>ovary</u> is normal in old animals. The
rupture of each follicle leads to the development of a corpus
albicans. This is simply a scar which remains as a result of
degeneration and fibrous replacement of the corpus luteum. With
increasing years these gradually accumulate and undergo hyaline
degeneration so that in ovaries of old cows the surface will be
seen studded with scars, the corpora albicantia. Primitive ova
become exhausted, or cease to develop into mature follicles. This
we speak of as senile atrophy, and representing as it does loss of
ovarian function produces sterility. The period in the animal's
life at which this change occurs is not definitely set as in the
human, for so far as is known animals do not pass through the
menopause. Sometimes the early development of senile changes
resultsin sterility from atrophy in young animals.

H. It is not infrequent to see sterility in cows, with
suppression or feeble estrum, irregular periods, or even complete
absence when on careful necropsy <u>no evidence</u> <u>of</u> <u>disease</u>, either
gross or microscopic, can be found in any organ. Some of these,
it is suggested as resulting from derangement of the endocrines,

others are possibly the result of reversion upon the part of the
animal to the wild state where estrum in the "Ungulata" occurs but
once a year.  The suppressed estrum may be an expression of the long
anestrous period common to the species before domestication.
Marshall[33] cites the observation, that wild monoestrus animals,
belonging to the same order in the kingdom as the bovine, when
placed in captivity change to polyestrus types, through change in
habit.  If this is so, reversion in the other direction, influenced
by physical factors, is also possible and may explain long continued
absence of estrum in some animals when no other cause can be adduced.

## Diseases and Affections of the Uterine Tubes

The importance of diseases and defects of the uterine tubes,
in relation to sterility cannot be overestimated.  It must be kept
in mind that the uterine tubes are the ducts of the ovaries and the
ova must pass through them in order to reach the uterus.  It will
be remembered that their lumen is narrow, their structure delicate
and their mucosa thrown into folds, directed toward the center, so
that injuries, although slight, may result in temporary or permanent
sterility.  One normal tube is all that is necessary for conception
but, as a rule, infections are bilateral, thus rendering both sides
impassable to ova.  It is said that simply the loss of cilia from
the epithelium is sufficient pathologic change to produce sterility.

A. Senile Changes, or atrophy of the tubes is normal in old
animals, (in women after the menopause).  The chief characteristic
of the change is atrophy, the tubes becoming smaller.  Atrophy
frequently results from pelvic adhesions, brought about by infections

and pelvic tumors.  It may also result from impaired circulation
secondary to ovarian cysts, or degenerative changes in the intima of
arteries and arterioles supplying the tubes.

    B. Circulatory disturbances are:

          1. Anemias, from local obstruction or a general anemic
               state.

          2. Hyperemias

               (a) Active, during estrum and resulting from
              infection.

               (b) Passive, occur as result of obstruction to
              return flow of blood, general congestion, throm-
              bosis of or tumors pressing upon the ovarian
              veins.

In active hyperemias due to infections we have the only circulatory
disturbance of any importance in sterility.  The typical picture of
inflammation is produced.  In passive hyperemias the tubes appear
blue, the typical picture of retarded outflow of blood.

    C. Hematosalpinx, the accumulation of blood in the uterine
tubes, occurs as result of tubual pregnancy, or twisting of the tube
resulting in passive congestion and tubal hemorrhage.  Such twists
may be produced by ovarian tumors and ovarian cysts.  It may result
in rupture of the tube with fatal hemorrhage, or the clot may under-
go organization thus destroying the function of the tube through
stenosis.  Tubal pregnancy is rarely seen in the bovine.

    D. Acquired displacements may be seen, as torsion or adhesions
of the tubes.  These frequently follow torsion of the uterus, ovarian
tumors or cysts, adhesions brought about by parauterine infections,
or other inflammatory reactions, such as produced by peritoneal

tuberculosis. Adhesions may occur without torsion and occlude the duct, thus rendering the lumen impassable to ova. In these, most often bacterial infections are the cause and the changes seen are for the most part similar to those described for chronic salpingitis. Many times as a result of ovarian cysts the tube undergoes torsion, near the ampulla. This results in occlusion of the duct, and the tubal and ovarian secretions (from ruptured cysts), are dammed up behind the stenotic portion. Thus by close adhesion to the ovary tubo-ovarian cysts are formed. The importance of displacements of the uterine tubes to sterility, lies only in the possibility of bi-lateral involvement.

E. Inflammations of the uterine tube are known as salpingitis. When involving the mucosa, they are designated endosalpingitis, the mucosa and muscularis simply as salpingitis, and when involving the serosa, perisalpingitis. It is rare that the mucosa alone is attacked, probably only in the early stages of inflammation is it seen independent of deeper involvement. Perisalpingitis may occur from within outward, or by extention from the abdominal osteum of the tube. Very frequently it follows para and perimetritis, with resulting adhesions and displacements as already described. Extention of tuberculosis may also be a cause. Salpingitis is by far the most frequent and important condition seen in the uterine tubes. It may be divided into acute and chronic catarrhal salpingitis, and acute, subacute and chronic purulent salpingitis.

Etiology. In the bovine, as in woman, it may be said that salpingitis occurs only as the result of bacterial infection, the most common invader in cattle being Bacillus pyogenes (Lucet), with streptococci and staphylococci usually present, but playing a

secondary rule. Salpingitis very seldom occurs except as a result
of puerperal infection, but may occur (as in one of our cases) by
extention of pyometra in a virgin heifer. The relation of B. abortus
to salpingitis is merely as a predisposing factor, preparing the way
for pyogenic infection of the uterus, with a secondary salpingitis.
It may rarely result from the extention of peritonitis or pelvic
abscesses. Theoretically, salpingitis may be primary and of
hematogenous origin, but practically, this never occurs. Salpingitis
is almost always bilateral

The results of salpingitis are, (1) permanent damage, and (2)
ho permanent damage to the tube. Its extention to the peritoneum
often causes peritonitis localized in the pelvic region with form-
ation of large abcesses, or generalized peritonitis. Fusion with
the ovary not infrequently results in the formation of large tubo-
ovarian abscesses. Then, too, it may heal in the incipient stage
and complete recovery result.

1. Catarrhal Salpingitis, occurs as a preliminary stage in
the course of inflammations of any mucous membrane and is most of
the time not the stage of inflammation resulting in serious damage.
It is a transitory outpouring of mucous seen in the congestive
stages of inflammation. Gillman[44] has described chronic catarrhal
salpingitis as producing productive inflammatory changes in the
tubes with resulting stenosis, and hydrosalpinx. It is probable
that organisms may be of sufficiently low virulence to produce
such changes, but it seems more likely that most of the chronic
changes result from old purulent infections. In catarrhal salpin-
gitis lymphocytes are the predominating cells in the inflammatory
exudate, rather than polymorphonuclear leukocytes. The process and

changes are similar to those described for chronic purulent
salpingitis.  It is a low grade chronic type of inflammation from
the start.

2. Purulent Salpingitis may be conviently divided into
acute, subacute, and chronic.  The subacute stage is an arbitrary
division to indicate the gradual progression into the chronic type.

The acute form is ushered in by the ordinary inflammatory
reactions;  congestion, edema and exudation.  As is true of
inflammatory reactions on all mucous surfaces the first change seen
is congestion, during which time there is a serous or catarrhal
exudate.  In case the infection is halted here, no further change
occurs, and the mucosa goes on to repair.  Healing either results
or the process proceeds to the next stage.  The increased
secretion (or catarrhal stage) is followed by polymorphonuclear
leukocytes, red blood cells, lymphocytes and plasma cells.  At first
few in number they gradually increase until the exudate appears
muco-purulent.  The clear mucous is streaked with pus.  Gradually it
becomes more purulent and contains the cells and debris ordinarily
seen in any purulent exudate.  The tube is swollen, and distended
with pus which has free drainage to the uterus.  In the beginning
the walls are stretched and thin, but if the process continues they
become thick through organization and proliferation of highly
vascular connective tissue.  This results in a tortuous or kinkled
tube.  Microscopically the lumen of the tube is seen to contain a
purulent exudate, consisting of inspissated serum, fibrin, poly-
morphonuclear leukocytes, a few lymphocytes and plasma cells,
bacteria and detritus.  The epithelium is irregularly degenerated,
due to the action of the bacteria, but always shows less involvement

than would be expected. The crypts between the mucous folds form pus pockets. Microscopic abscesses may be seen in the mucosa and tunica propia, even extending to the muscularis coat. If healing is to occur and leave an unimpaired tube it must take place at about this stage. If healing does not result the whole affair gradually passes on through subacute and chronic stages from which recovery of function is impossible.

The chronic stage, the one almost always seen at autopsy, is dependent upon the continued progress of the infection. Bacteria invade the deeper structures of the tube producing greater kinking, and greater thickening of the walls. Marked changes appear in the mucosa. The epithelial folds, ordinarily quite delicate, become greatly hypertrophied and often adhere to one another. Later actual growth from one to another and across the lumen occurs, and with the gradual distention of the tube they continue to grow, appearing as thin-walled, small multilocular cavities. The epithelium is yet, for the most part, intact. The exudate gradually subsides and in place of polymorphonuclear leukocytes as seen in the acute stage, the predominating cells seen in the exudate are lymphocytes and plasma cells, with much fibrin. Organization or lysis of the exudate usually begin at this stage. In case of the former the process may progress slowly with gradual diminution of the exudate and progressive organization. The walls become permanently thicker, the mucosa may entirely disappear or the mucous folds through fibrosis may become obliterated and thus contribute to the thickening of the wall. Many times this leaves the base of the folds below a solid fibrosed area, as remnants of tubal epithelium. In cross-section these remnants appear as acini of glands. This process may continue to complete stenosis of the duct, with

disappearance of the exudate. It often happens that the rate of proliferation and organization occurs more rapidly at one point than another, probably aided by kinking of the tube with stenosis. The favored site for early stenosis is the abdominal osteum with later stenosis at a point below the ampulla. As a result of this stenosis the exudate collects and distends the tube. The adherent mucous remnants become stretched but are usually sufficiently strong to retain their attachments. The dammed off portion of the tube in this state of pus distention is known as pyosalpinx (pus tube). It seems that in cattle the distention is never as great as is seen in salpingitis in woman, for rarely are the adherent mucous folds ever pulled apart or separated and flattened out, but retain the multilocular character. The microorganisms gradually die or are already dead at this time. The pus is gradually absorbed and re- placed by a clear, watery, light, straw-colored transudate, or later by mucous secretion from the regenerated tubular epithelium. The fluid contains but few leukocytes. Bacteria may be found present from the active inflammatory process which preceded, but if careful cultural methods are employed the fluid will usually be found sterile. This last type or stage we designate hydrosalpinx. We are not always sure that salpingitis proceeds as here pictured, but the changes seen bespeak of previous active infection for all transitions can be seen between the infective stage and the final. The typical appearance of hydrosalpinx in cattle is a large thin- walled tortuous tube, containing a clear fluid. The lumen is marked off by adherent and stretched mucous folds, appearing as small multilocular cysts. Each fold is supported by its basal membrane and lined by quite normal appearing epithelium. It is possible by

sufficient distention of the tube to have the flask or retort
shaped pyo and hydrosalpinx as seen in women, but we have observed
this form only once. Dr. W. L. Boyd has one of this type in his
collection. Frequently at autopsy only the ampulla will be seen
involved. It seems that the uterine end of the tube very often
heals but leaves a stenotic ampulla. The ampulla then passes on to
chronic salpingitis ending finally in hydrosalpinx and leaving the
uterine end of the tube unimpaired. Sometimes the stenosis occurs
near the isthmus in which case the entire tube passes on to chronic
salpingitis. The results of salpingitis after the acute stage is
passed and the chronic stage begun can always be regarded as
productive of incurable sterility, in so far as cattle are concerned.

F. Of the specific infections tuberculosis and actino-
mycosis are the only two involving the uterine tubes. Grossly
either may appear like a pus tube, or purulent salpingitis, but are
easily differentiated microscopically. Tuberculosis is nearly
always secondary to an already existing focus of infection, while
actinomycosis is more often primary in the genital organs. In
tuberculous salpingitis the tube is swollen and reddened, and pus is
found in the lumen. In chronic cases the tubes thicken and become
firm and filled with caseous exudate, containing inspissated serum,
a few polymorphonuclear leukocytes, plasma cells and many lympho-
cytes. Miliary tubercles with or without calcification develop
throughout the walls. The reaction involves the mucous folds which
become adherent by organization and extention of the tuberculous
process and often are entirely replaced by the tubercles and
caseation necrosis. In milder cases remnants of epithelium will be
seen deep below the newly formed tissue, in this respect appearing

oftentimes like acini of glands without outlet to the surface.
Sometimes in chronic cases the tubes appear beaded through twisting
and bulging of the walls, caused by the developing tubercles.  The
outcome of tuberculous salpingitis may be tuberculous peritonitis,
tuberculous metritis, extention to the ovary, generalized miliary
infection from invasion of blood stream, pyosalpinx (pus tube), or
a chronic tuberculous salpingitis without healing, but usually with
occlusion of the tube.  As tuberculosis is usually bilateral,
permanent sterility is in most cases an inevitable outcome.
Actinomycosis when developing in the uterine tubes causes extensive
pelvic involvement through extention, by the development of fistulae.
It is similar in appearance to actinomycosis elsewhere, and invariab-
ly results in permanent sterility of fatal termination.

G. Tubal gestation has been reported in the cow but is so
very rarely seen that only passing mention need be made.


## Diseases and Affections of the Uterus

The uterus is of all the genital organs, the essential one for
the development of the embryo.  It is often the seat of changes or
infections which render the animal temporarily or permanently
sterile.  The congenital anomalies have been previously mentioned.

A. Acquired anomalies and deformities are not commonly
seen.  They may develop from pelvic and intestinal adhesions follow-
ing infection, or as the result of tumors of the genital tract or
broad ligaments.  Torsion of the uterus quite frequently occurs, the
cause of which, theoretically, may be similar to those listed above,
but it almost always accompanies gestation.  Rupture of the

ligamentous attachments of the uterus may render the cow unfit for further pregnancies. Prolapse of the uterus may follow such an accident, but usually results only from dystocia where severe straining and traction are the exciting cause. Lacerations and perforation occur and are usually accidental from obstetrical or surgical manipulation. If peritonitis does not result, these are not ordinarily serious. Loss of cotyledons (carunculae) occurs usually following manual removal of the placenta or following prolapse, but does not as a rule, render the animal permanently sterile. It seems rarely to occur.

B. **Circulatory** **disturbances**, such as **anemias** and **hyper-** **emias** (active and passive), are seen. Active hyperemia is physiological in estrum and gestation, and occurs pathologically in inflammations. Passive hyperemias develop as in the uterine tubes; neither in themselves are important contributary factors to sterility. Anemias may occur as described for the uterine tubes.

C. **Inflammations** are spoken of as metritis, and may conform to the following arbitrary classification, based upon clinical and pathological findings.

    1. **Acute** **metritis**,-**mild** or **severe**.

        (a) Puerperal infection.
        (b) Non-puerperal infection.

    2. **Chronic** **metritis**.-

        (a) Begins as a low grade infection and continues as
            such.
        (b) Develops from acute (a or b) by long continued
            infection.

Some workers further subdivide into acute and chronic endo-metritis, perimetritis and parametritis. These terms refer simply to location of the inflammatory process, but from the pathological

viewpoint can hardly be said to exist as independent disease
processes. One type blends closely with the other, so intimate are
the anatomical relations. The term metritis will be used in the
broad sense with the other terms as descriptive of location.

Etiology. Metritis in every instance develops as a result of
infection. The predisposing causes may be injury, through trauma or
irritating fluids but these only make possible the invasion by
bacteria. Eggink[45] in an analysis of 20 cases of acute and chronic
metritis found the following organisms:

| | |
|---|---|
| B. tuberculosis | 2X |
| B. pyogenes bovis | 14X |
| Streptococci | 12X |
| B. coli | 6X |
| Staphylococci | 5X |
| B. proteus | 3X |
| B. subtilis | 1X |

In many of these, mixed infections were present. From analysis of
cases the most important organisms appear to be B. pyogenes, and
streptococci. Except in one case of tuberculous metritis, either
one or both of these organisms were always found. Lucet[46] first
described B. pyogenes bovis (1893), when he studied 52 cases of
suppuration in cattle. In these he found B. pyogenes bovis pure in
10 cases and associated with other bacteria 7 times. His type has
since been found identical with B. pyogenes suis (Grips) and the
name B. pyogenes has been proposed for both. Künnemann[47] studied
many cases of suppuration in cattle and concluded B. pyogenes the
most important etiologic agent. He observed this organism in 90%
of cases, in 35% pure and in 55% associated with other organisms.

In a survey of 18 cases (purulent, acute and chronic metritis),
reported by Wall[16], the following organisms were found:

| | |
|---|---|
| B. pyogenes | 6X |
| Streptococci | 4X |
| Streptococci and B. pyogenes | 5X |
| Streptococci and B. coli | IX |
| Streptococci, B. pyogenes, & B. coli | IX |
| Streptococci and an anaerobic bacillus | IX |

Brown and Orcutt (1920)[48] in their "Study of B. pyogenes", worked
with twelve strains of the organism, five of which were isolated
from cases of purulent metritis. The results of the present
investigation, which will appear in detail later, confirm the
importance of B. pyogenes in bovine metritis.

1. Acute metritis of the mild type generally involves the
endometrium alone and thus in mild cases, acute catarrhal endo-
metritis is frequently seen. It is nearly always the result of an
infectious abortion. However, it may follow retention of afterbirth
of full term gestation, but more often the latter type is more
severe. Following the infection of the uterus and placenta by
B. abortus an inflammation is set up, wnich results in the premature
delivery of the fetus. This organism is not capable of establish-
ing itself in the normal involuted uterus and soon disappears after
the abortion. If no secondary infection follows, only a mild acute
catarrhal endometritis will ensue, marked by excessive discharge,
muco, and mucopurulent, which as involution takes place will rapidly
disappear without permanent changes remaining. The superficial
lesions seen in the mucous membrane are necrosis and exudative
inflammation. The former is a normal accompaniment of involution

in the physiologic discharge of the fetal and maternal placenta.
Healing in most cases is complete, but Wall[16] states that shrinkage
and atrophy of the gland mucosa may occur, with probably similar
changes in the carunculae, - this change results in no permanent
damage to the organ.

2. Acute metritis of the severe type is in the vast majority
of cases puerperal in origin (involving endometrium and often other
uterine coats). Death and maceration of the fetus in utero is
frequently a contributing cause, or it either follows infectious
abortion or full-time parturition.  In either case retained
placenta favors the infection, or it may be introduced from the
exterior by the operator or accidentally from the vagina.  In the
majority of non-puerperal infections it follows injury, often
during breeding, or through extention of cervical and vaginal
infections.  In case the retained placenta follows an infectious
abortion the retention results from premature delivery.  The
placental attachments remain adherent and are removed only with
difficulty.  In the case of retention following full-term delivery
the etiology of the retained membranes is not clear.  The effect of
the abortion bacillus on the uterus, in case there is no retention
of the placenta, is simply to cause a mild catarrhal endometritis.
Where the placenta is retained, and this applies to all forms of
retention, the involution of the uterus is delayed. Organisms,
both parasitic and putrefactive types, enter through the vagina.
The mucosa is not able to resist their entrance and severe purulent
metritis develops. The state of the mucosa, following delivery, it
will be remembered, is deprived for the most part of its epithelium.
Carunculae slough off in part, in order to release the fetal

chorionic villi. Other cases develop following dystocia, especially

when operative procedure is not properly applied, or simply as

accidental infections when the utmost care is exercised. The

organisms most often associated with this type of infection are

streptococci and Bacillus pyogenes. Wall[16] makes the observation

that if the infection be purely due to streptococci the process

heals quite rapidly but when resulting from B. pyogenes either pure

or associated with other bacteria, pyometra is the rule. The work

to be presented in this paper supports this view.

Pathologic changes. The endometrium shows necrosis of varying

degrees (partly physiological following parturition). It is usually

diffuse. Congestion and edema develop coincident with exudative

phenomena. At this time large quantities of mucous lie in the

cavity of the uterus. Polymorphonuclear leukocytes are soon

attracted in great numbers and form a considerable portion of the

exudate. The leukocytes gradually increase in numbers until the

exudate is decidedly purulent, appearing as thick creamy pus. As a

result of the action of putrefactive bacteria, decomposition of the

pus sometimes is noted. Bacillus proteus, anaerobic bacilli, and

other protelytic soil types are responsible for the offensive odor.

This has nothing to do with the progress of the infection. In the

mucosa there may be seen large numbers of polymorphonuclear

leukocytes, lymphocytes, eosinophiles and plasma cells, often

collected in groups to form microscopic abscesses, (not to be mis-

taken for the normal lymphoid tissue of the uterus which is also

accentuated as a result of disease). These even may extend to the

muscularis and serosa, but not as a rule. The gland epithelium

becomes necrotic and in many cases lost;  other glands are blocked in

their course by proliferative changes and appear as small cysts.

The epithelium of the mucosa generally regenerates but is not a healthy type, neither is it entirely regenerated during the course of the infection. It is surprising in studying cases of purulent metritis to see the uterus in as healthy a state as one invariably finds it. The changes in the wall are in no way commensurate with the exudate in the lumen. This type heals early or passes on to the subacute and chronic types.

Symptoms produced are those of general sepsis, as anorexia, slight fever, stupor and cachexia. As a rule, these symptoms are not marked unless retroperitoneal abscesses develop (localized peritonitis).

Acute metritis may continue to chronic metritis, with development of salpingitis, perimetritis, abscesses of the parametrium (localized peritonitis), ovarian abscesses or generalized peritonitis with fatal termination. Thrombosis of veins in the vaginal plexus may occur. In other cases it may continue to pyometra as a subacute inflammation, uncomplicated for several weeks, and result in complete recovery when properly treated. Then too, the condition may have a favorable termination in the incipient stage (especially when of the Streptococcus type).

3. Chronic metritis. In most cases after an acute attack the purulent discharge seems to subside and the observer will think the case healed. This is an expression of closure of the cervical canal. In many cases, the uterus fails to undergo involution following parturition (subinvolution of the uterus) and throughout the acute attack remains in the evolutionary stage. It gradually fills with the purulent exudate already described, until possibly 5 liters collect. It then begins to ooze from the vagina when the animal is decumbent.

This damming up of the exudate we speak of as <u>pyometra.</u>  There
appears to be in cases of infected uteri, together with failure of
the uterus to undergo involution, also the failure of the corpus
luteum to disappear (persistant corpus luteum), exemplifying the
close physiologic relationship existing between these two organs, -
ovary and uterus.  In other cases free drainage is continuous with
apparently the same uterine changes, simply a purulent metritis.
The course is similar to that already described in acute metritis,
and the complications there described are more often delayed until
the chronic stages.  Recovery in simple purulent metritis without
complications, under proper treatment, is often noted.  Pathologic
changes are similar to those already described for acute metritis,
simply more pronounced.  The proliferative changes are accentuated,
as also are the changes in the gland mucosa.  Wall[16] describes
regeneration of the normal uterine epithelium by ectodermal types of
epithelium as seen in the vagina.  This he calls "ectodermoisering."
He regards this as a permanent change, affecting the usefulness of
the organ.  The fibrous tissue is accentuated by proliferative
changes and in the muscularis, Wall[16] observed atrophic changes,
other times hypertrophy depending upon the type of infection.   The
fibrosis may be so severe that the uterus becomes very hard and
thick.  In this state the organ is called sclerotic.  We have studied
and Dr. W. L. Boyd has treated several cases of chronic metritis with
excellent results, apparently complete recoveries have occurred.  It
will be interesting to follow these cases to see whether changes
exist which will render the animal sterile.  It is possible that
even though no palpable lesions exist there are physiologic
disturbances, producing altered secretion of the glands.  The normal

uterine secretions in order to support fertilization of the ova must be neutral or faintly alkaline. Spermatozoa are killed in acid secretions. Such abnormal secretions are well-known in women and probably give rise to many cases of sterility in the human female. If such changes occur in the uterus of the cow no doubt they exert a similar action on the spermatozoa. Since there is a successful treatment for such conditions (alkaline douches) an investigation of this should be continued when available material presents itself. Hallman[61] in a report on the pathologic findings in eleven cases of bovine sterility observed alterations of the uterine mucosa, varying from slight fibrous thickening of the transverse cervical folds with no apparent alterations of the corporal and corunal mucosa to an atrophic endometritis. "The lesions observed in varying degrees in the different cases are mucoid degeneration of the superficial epithelium, local and diffuse fibrosis of the uterine mucosae, leukocytic infiltration of the stroma and gland luminae and degeneration and disintegration of the glandular epithelium with dimunition in the number of glands. In the majority of cases the anatomical alterations are comparatively few and it is hardly conceivable that failure to breed was the result of loss of functional tissue of the uterine mucosa." The studies in this investigation support this view.

4. _Chronic catarrhal endometritis_ is a clinical subdivision of ordinary chronic metritis and is used to designate a chronic catarrhal metritis, in which there is little formation of purulent exudate. The infection begins usually following abortion or full-term parturition and continues as a low-grade inflammatory process, causing increased secretion and hypertrophy of the mucosa. Cystic

and large tortuous glands are often seen.     At other times they are

atrophic.    There is frequently a mucoid degeneration of the mucus

secreting glands.    From reliable sources the information has been

obtained that 90% of curettements from women, in which curettage

has been done for chronic catarrhal endometritis, are normal and

that the symptoms observed are the result of altered ovarian

function, in most cases, with no evidence of an inflammatory endo-

metrium.    It is better to regard these cases, when no active

infection can be demonstrated in the endometrium and where pathologic

changes exist, as not active inflammations but the result of acute

or chronic metritis as already described.

    D. Hypertrophic endometritis is occasionally seen in the

bovine.[62] Dr. W. L. Boyd at the University of Minnesota has one such

case in his collection.  The uterine mucosa in these cases becomes

much thickened.  It becomes very vascular and quite susceptible to

hemorrhage.  The glands enlarge and become cystic and on the

surface of the mucosa there develop irregular rounded polypoid-like

projections.  Many of the cases diagnosed as hypertrophic endo-

metritis, in human practice, have simply been physiological changes

of menstruation.  There is no doubt though, that this type of

disease exists.    It results in incurable sterility, for in the

bovine curettage is not practiced.  The cause is unknown, but by

many it is regarded as inflammatory.

    E. Cystic degeneration of the uterine walls with hydrometra

is described by Williams.[62] It is the outcome of many pyometra cases,

all severe cases being more or less affected.    Dr. W. L. Boyd has

one such case of severe type in his collection.  The uterine glands

become cystic, and the uterus contains much clear fluid.  The

mucosa grossly appears studded with cysts. Microscopically large
dilated uterine glands are seen. It results from inflammation
a termination of chronic metritis.

F. Specific inflammations are produced by B. tuberculosis
and Actinomyces bovis and are essentially the same as infections
produced elsewhere by these organisms. Sterility with systemic
complications usually result, or else these infections are
secondary to some more generalized lesion already existing.

G. Hematometra, distention of the uterine cavity with
blood is rarely seen in the bovine. Williams describes these
cases and states that not infrequently the clot may undergo
organization and result in a large uterine hematoma.

H. Tumors of the uterus, according to Williams[26], are
rare. From comparative pathology leiomyomata should be the most
common, with adenomata and carcinomata second. Chorion epitheliomata
and hydratid moles are not reported for the bovine. Tumors when
present result in sterility, especially when rapidly growing or
large.

## Diseases and Affections of the Cervix

As no especial attention has been directed toward the
consideration of cervical and vaginal diseases which may lead to
sterility, only a brief résumé of these will be given in this
report.

A. Occlusion of the os uteri, with a cervical canal so
tightly closed that spermatozoa cannot enter, is occasionally found
in cows.

B. Dilation of the cervix uteri, through loss of muscular

tone, is occasionally seen and often the atony is so great that

conception cannot occur. It is an accompaniment of atony of the

uterus in nearly all cases.

C. Hypertrophy of the os uteri externum frequently occurs,

as a result of low grade proliferative inflammations following

injuries at parturition, through use of instruments or from

extention of vaginal infections. Grossly the folds show great

hyperplasia so as to partly block the cervical canal. The mucosa

is hyperemic, and often covered with small red papules and vesicles.

The epithelium is usually thickened and edematous, while, as a

result of rupture of vesicles, it may be desquamated in some places.

Sterility is sometimes an accompaniment, but only temporary until

surgical relief can be applied unless as is frequently the case,

the hypertrophy is occurring with a severe B. pyogenes or

Streptococcus cervicitis and metritis.

D. Inflammations of the cervix and external os uteri often

occur as the result of irritating douches and unless infection

supervenes they are only transitory. Infections frequently follow

injury at parturition, or from use of surgical instruments. They

also may develop from extention of metritis and vaginitis, or may be

primary at any time. Such infections usually develop slowly with

exudation and proliferation and probably are in most cases caused

by streptococci or Bacillus pyogenes. The arrangement of the

cervical folds in plaits of mucosa, serves to make the infection an

obstinate one and difficult to treat. The most frequent compli-

cations are hypertrophy of the cervical folds, adhesions resulting

in atresia, and extention of the infective process to the uterus

and other genital organs. Increased tenacious secretion may result

and lead to sterility through mechanically entangling the spermatozoa
before they gain entrance to the uterus. This produces a temporary
sterility, and in human practice has been found quite ammendable to
treatment (saline douches). Sterility is present during the
infection, but if it subsides before pathologic changes are great
judicious treatment usually effects a cure.

E. Tumors, such as polypi are reported, but these growths
are rare and seldom malignant carcinomata are not reported as
occurring in the cervix of the bovine.

## Diseases and Affections of the Vagina

A. Congenital abnormalities: The persistence of the hymen
into adult life is occasionally seen and leads to sterility until
operative removal. Sometimes a small opening is noted, while in
other cases none exists. Bands are sometimes seen representing the
embryonic median walls of the fused Müllerian ducts, which have
failed to atrophy and disappear completely. They may persist so
completely so as to constitute essentially a double vagina
(Williams)[26]. They can, as a rule, be reduced by surgical procedure
so that permanent sterility is not a result. The almost complete
absence of vagina in freemartins and in other anomalies and arrests
in development of the genital tract have been mentioned.

B. Acquired Anomalies and Defects: Between the walls of the
vagina, as the result of proliferative changes following infections,
strong connective tissue bands frequently develop resulting in
atresia of the vaginal walls. One such case is included in the
case reports, which follow. The stricture when extensive renders

the animal sterile, when slight, surgical interference results most
often in recovery.

C. Hypertrophy of the vaginal walls is frequently seen,
occurring during the late stages of gestation and in the non-
pregnant animal. The wall of the vagina, 10-12 cm. anterior to the
vulva, laterally and dorsally, become hyperplastic so that partial
prolapse of the vagina results, ordinarily seen only while the
animal is decumbent. Severe inflammatory changes sometimes develop
and result in more complete prolapse, induced by straining from
the irritation. The condition is ordinarly successfully treated
through surgical removal of the hyperplastic portion of the wall.

D. Prolapse of the vagina is seen in cases other than from
the development of hypertrophic walls. It is occasioned by late
pregnancy, especially in fleshy animals, and from injuries following
parturition. The prolapse is frequently not complete, and visible
only while the animal is decumbent, but may be severe and complete.
It nearly always responds to treatment and when healed produces no
unfavorable results.

E. Vaginismus (violent spasms of the vagina) are frequent
symptoms of diseases of the genital tract and ovaries. They
influence conception and are relieved when the cause is removed.
Their influence upon sterility of the animal is only secondary.

F. Cysts of Gartner's ducts (remnants of the Wolffian duct)
may interfer with successful breeding but do not result in absolute
sterility.

G. Cysts of Bartholin's duct are described by Hess[49] and
Hofman[50], the glands become hyperplastic and until surgically removed
prevent proper conception.

without inflammatory reaction in the vagina. The vagina because of
its stratified epithelial surface, is not prone to become infected
unless preceded by mechanical injuries anymore than is the external
surface of the body. No study has been made of vaginitis but
probably B. coli and staphylococci are more often the etiologic
agents than are streptococci or B. pyogenes. The pathologic changes
are similar to those seen on any mucous membrane, and are in most
cases only acute, accompanied by a mucopurulent discharge.

I. Tumors, such as polypoid masses and papillomata, are
sometimes seen but are not common. Carcinomata and sarcomata are
rarely seen in this location, in the bovine. Williams states that
carcinoma of the vulva is not rare in the cow.

J. Diseases of the Pelvic Bones, although rarely seen,
when present are productive of sterility in that the animal can no
longer carry the fetus properly. Fracture of the ileum thru injury
may be responsible for this condition. Osteomalacia may render the
pelvic bones friable and result in narrowing of the pelvic inlet to
such an extent that parturition is rendered difficult or impossible.
So-called dropping of the tail head (sacrum) may produce a similar
result. In nymphomania (cystic ovaries) the tail head quite often
becomes raised through relaxation of the pelvic ligaments. Misplaced
genitals may result, but is returned to normal when the cause is
removed.

## The Present Investigation

### Methods of Study

The object in view, during the progress of this investigation,
has been to obtain cases which were diagnosed by clinical procedure,
as sterile. Sterility in some instances was considered as absolute,

and cases falling into this group were slaughtered, and the genital organs studied both pathologically and bacteriologically. Clinical history, although not always complete, is available in all cases of this group. Other animals were presented at the University clinic for treatment of sterility, the majority of which responded favorably to the clinical procedures employed. Improvement in this group was apparent, with sugestion of recovery. Since the affected animals were valuable purebred stock, slaughter was not advised. In these cases bacteriological studies combined with clinical data, constitute the report. When possible cultures were obtained from the genital tract (uterus) during the progress of the infection, this was done by inserting sterile swabs through the cervical canal, into the uterus. The same method was employed in all cases where the genital tract was cultured during the life of the animal. At necropsy the genital organs were carefully removed, and cultured systematically. The organs were seared at the point of study and penetrated with sterile instruments. At the point of penetration, material for cultural study was obtained. Cultures were prepared on 10% sterile horse serum agar and the tubes sealed off with hot wax. These were allowed to incubate from 10 days to 2 weeks at 37°C. By this method anaerobic types, such as B. abortus could develop. The aerobic cultures were prepared by the plate method, using agar in combination with sterile defibrinated horse blood. By this method streptococci and B. pyogenes were most easily obtained in pure culture. As soon as possible after cultures were prepared, sections were taken from various parts of the genital tract, Preservation and fixation was accomplished in Zenker's solution, and in 10%

Formalin. Sections were run by the paraffin method, and also by the freezing method. Hemotoxylin and eosin were employed in the staining technique. Cultures were identified individually, in all instances the most modern and also original references were consulted. For Bacillus pyogenes (Lucet) the original work of Lucet[46], Künneman[47], Grips[53], Glage[54] and Brown[48] were consulted. For the work on streptococci the works of Brown[58] and Hollman[57] have been used exclusively. The work of Winslow et al[56], has been followed in studies upon the staphylococci. In all 19 cases are reported, only one is without clinical history, this being a tuberculous metritis and salpingitis obtained at the abattoir.

## Case Reports

### Case 1

Clinical History Jersey cow, age 5 years. First calf born at two and one half years of age, full term but born dead. Agglutination reaction for infectious abortion, positive up to 1-200 May 1918. Second calf aborted June 1918. Received into University experimental herd December 1918. Bred January 27, 1919; examined and pronounced pregnant March 6th; examined again in April, at which time found non-pregnant. It is assumed as reasonably certain that she aborted between first and second examination for pregnancy. Bred April 14, May 25, and June 24, 1919. The animal never gave evidence of any extreme signs of heat, coming in and going out normally. Bred again July 5, 1919, July 29, 1919. Allowed to rest for several months. Uterus examined and massaged without detecting any abnormality. Bred October 18, 1919, November 28, 1919, January 22, 1920 and February 5, 1920. Examined again March 15, 1920, and found to be

non-pregnant, genital organs apparently normal. Agglutination reactions of blood to B. abortus antigen, after the animal entered the University herd were as follows: November 1919, and March 1920, positive 1/20 to 1/1000 and inclusive dilutions. The history of the animal indicates the prevalence of sterility in the herd from which she came. The mother was difficult to get with calf, but never aborted. After parturition there was usually retention of the placenta. One sister of Cow case 1, was sold for beef, because of sterility, and a second one was recently sent to the University herd because of frequent abortions (last 3 calves aborted). The animal's condition (case 1) was considered incurable, and for this reason was slaughtered March 30, 1920.

## Necropsy

1. Gross Description. The general condition of the carcass is good. The vagina is normal. The cervix is normal. The uterus appears normal. The tuba uterina appear normal. The right ovary is 3.5 cm. in length, and 2 cm. in width. It appears to be more adherent than normal to the broad ligament. It contains a corpus luteum 8 mm. in diameter. The left ovary is 2.7 mm. long, 1.3 mm. in width. It contains a persistent corpus luteum deeply buried in its stroma. The corpus luteum is 2.5 cm. in diameter. The center of the corpus luteum presents a cystic cavity 1 cm. in diameter. The ovarian tissue is greatly flattened over this retained and cystic corpus luteum.

2. Microscopical Description. The vagina and cervix uteri are normal. In the uterus some of the innermost glands show degenerative changes. The glands are enlarged, and the epithelium of many is

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partly degenerated. In the lumina there is an exudate containing desquamated cells and mucus. The nuclei of the gland cells are pyknotic. All of the deeper lying glands are normal. The stroma of the gland mucosa is less cellular than normal, due to increased connective tissue proliferation, mostly collagenous fibrils. The other coats of the organ are normal. The uterine tubes are normal. There are many primitive follicles in the cortex of the right ovary. Lying deeper in the ovarian cortex there are three follicles (in one portion of the ovary), showing atretic degeneration. The theca folliculi of these follicles is greatly thickened, and is very cellular. The membrana granulosa of the smaller follicles is proliferated so as to almost completely fill the central cavity. In the larger ones these cells are increased in number, many of which are detached from their normal attachment and lie free in the central cavity. Many of these cells have pyknotic nuclei and their cytoplasm shows a hyaline granular degeneration. Figure 1, illustrates this condition. Corpora albicantia are numerous throughout the ovarian stroma. Several normal nearly mature Graafian follicles are present. Other sections appear normal.

3. Bacteriological Findings:

1. Cultures from the uterus, uterine tubes and ovaries remained sterile.

2. Three types of organisms developed from the vagina. (a) Staphylococcus aureus (Rosenbach); (b) B. coli communis; (c) B. subtilis.

4. Diagnosis and Discussion. The etiology of sterility in this case is not clear. Possibly the deep seated cystic corpus luteum, which was present in the left ovary was a contributing factor. The

degenerative changes described in follicles in the right ovary may also have influenced the progress of the condition. The changes in the uterine glands may also have been sufficiently pronounced to inhibit implantation of the developing embryo, or to inhibit passage of spermatozoa to the ovum. The most probable change responsible for sterility is that described for the ovaries.

## Case 2

Clinical History. Shorthorn heifer. Bred for first time April 3, 1920. Appeared in estrum again April 26th, and was rebred to same sire. Immediately after this service the heifer was noticed to be suffering considerable pain. At termination of next diestrum, heat did not appear, but pregnancy was not suspected on account of a fairly copious and constant vaginal discharge. The discharge was of a mucopurulent nature, yellowish gray in color and had a pronounced offensive odor. The discharge also at times was streaked with blood. A physical examination of the vagina revealed severe though not active inflammatory changes. The mucous membrane of the vagina was studded with small vesicles, such as characterize granular venereal disease. Cervicitis was pronounced. The os uteri externum was covered by a profuse mucopurulent exudate. The cervical canal was partially obstructed by inflammatory changes. A rectal examination was made with considerable difficulty due to formation of massive adhesions between the uterus, ovaries and walls of the rectum and colon. This resulted in constriction of the lumina of this portion of the intestinal tract. The right ovary was found to be enlarged and adherent to the right uterine horn. The right uterine tube was sufficiently distended to render it easy to locate and was adherent to the ovary. The left ovary contained a

large cyst. It was enlarged and irregular in outline and adherent
to the left uterine horn. The left uterine tube was not definitely
located. The body and horns of the uterus were difficult to
palpate, but were thought to be somewhat hardened and thickened. A
diagnosis of incurable sterility was made and the animal was
slaughtered for food purposes.

## Necrospy Findings

1. Gross Description. The physical condition of the carcass is
good. The vaginal mucosa appears practically normal. The corpus
uteri is indurated, and adherent by fibrous attachments to the
rectum..No macroscopic changes are apparent in the endometrium.
The cervix uteri is inflammed and the cervical canal is more or
less obliterated, and tightly contracted. The cornua uteri appear
normal in gross structure. In the lumen of the uterus, throughout
its entire length, there is present a considerable mucopurulent
discharge. Some areas show pus undergoing caseation without marked
foul odor. The uterine tubes are dilated to 6 mm. throughout their
entire length. The walls are thick and fibrous. At the isthmus the
dilation forms a definite pus pocket being 2 cm. in diameter and
extending upward on the tube 3 cm. The fimbriated ends are adherent
to the broad ligament. In the lumen there appears a mucopurulent
exudate. This purulent material is odorless. The left ovary is
firmly adherent to the uterus by fibrous attachments which extend
from the body to the horn. It averages 6-8 cm. in diameter. The
capsule is slightly thickened. On its inner border, there is a
large persistent corpus luteum. The central portion of the ovary is
cystic. The cyst measures approximately 4 by 5 cm. in diameter.
The right ovary is very irregular in outline, and is comparable in

size to the left. A sagittal section shows only a small amount of normal ovarian tissue. Centrally located and surrounded by ovarian tissue, is an abscess the content of which is greenish-yellow and pasty in consistency. The pus is putrid, having a state pungent odor. The ovarian tissue forms a wall 1¼ cm. thick around the abscess. At the inner periphery and separated from the abscess by a slight constriction and wall of connective tissue, is a small cyst containing a straw-colored serous fluid. The cyst is one cm. in diameter, oval in shape and enveloped by a hard, but thin fibrous wall.

2. Microscopical Descriptions.

The endometrium of the uterus shows a partially exfoliated epithelium, only shreads remaining in which the nuclei are barely visible. That which remains intact is normal except for the frequent presence of leukocytes, and is composed of a simple columnar epithelium. Many uterine glands are cystic. The cysts are from 3 to several diameters larger than the normal glands and are made up af an outer wall composed of dense connective tissue fibers. These support, in a few places, a single layer of epithelial cells of low columnar type. Degeneration of this epithelium is pronounced. In the cystic cavity very little cellular material appears, only a few polymorphonuclear leukocytes and mononuclear leukocytes and a small amount of fibrin can be seen. Similar inflammatory cells extend into the cystic wall. Uterine glands in the vicinity of those which are cystic show atrophy, due to an overgrowth of connective tissue. This also appears in other areas where no cysts are seen. The epithelium of many glands is entirely atrophied and replaced by proliferations of young connective tissue cells. The stroma in

general shows a high degree of inflammatory reaction, there being
quite universally present, polymorphonuclear leukocytes, lymphocytes,
plasma cells and fibroblasts. In some areas these are collected so
as to form small abscesses. The myometrium shows similar but less
extensive cellular infiltration. The serosa is normal. The uterine
tubes show a normal serosa. The muscular coat is atrophied at the
expense of connective tissue proliferations. The epithelium of the
mucosa is simple columnar, and is exfoliated in some areas. Where
exfoliation appears the cells are degenerated and only a homogenous,
eosin staining, fibrin-like material remains. In the tunica
propria, there are masses of polymorphonuclear leukocytes closely
packed so as to form small abscesses in the wall. The lumen contains
dense masses of leukocytes, mostly polymorphonuclear, and small
mononuclear cells, among which plasma cells are occasionally seen.
Some fibrin is also present. Necrosis of the inflammatory elements
has commenced in the central portion. The medulla of the right
ovary consists for the most part of fibrous connective tissue.
Several areas show collections of leukocytes mostly polymorphonuclear
and mononuclear cells. In some places these form small abscesses.
In the cortex the stroma is very vascular. In one section there
appears an atretic follicle. One section shows a cystic follicle,
with the theca folliculi greatly thickened. Only remnants of the
stratum granulosum remain. Many of the nuclei of this layer are
pyknotic.

    3. Bacteriologic Findings.
        (1) Uterus and uterine tubes (a)Staphlococcus epidermidis
                                                    (Gordon)
                                (b)B. coli communis.
                                (c)B. pyogenes (Lucet).
                                (d)Streptococcus Mitis.(Alpha
                                                     type)

2. <u>Right</u> <u>Ovarian</u> <u>abscess</u>.

    (a) Staphlococcus aureus (Rosenbach)

    (b) <u>Streptococcus</u> <u>mitis.</u>

3. <u>Right</u> <u>Ovarian</u> <u>cyst</u>.

    (a) Sterile

4. <u>Diagnosis</u> and <u>Discussion.</u> 1. Purulent cervicitis, metritis and salpingitis. 2. The uterus shows evidence of chronic changes, such as fibrosis and cystic glands in the endometrium. 3. Right ovarian abscess, with chronic inflammation of right ovary. 4. Small follicles showing cystic and atretic degeneration are also seen. 5. Corpus luteum cyst of left ovary. These change support the diagnosis of incurable sterility. Infection most likely entered through injury at time of breeding and gradually proceeded to involve the entire genital tract.

## Case 3.

<u>Clinical</u> <u>History</u>. Beauty Frances, Holstein cow, age 2 years, delivered a dead full term calf following severe dystocia September 23, 1920. Traction necessary to make delivery. Placenta came away normally. Developed severe purulent metritis within five days after parturition. Parauterine abscesses soon developed, the larger of these being located on the superior surface of the vagina between the left side of the uterus and the rectum, in the pelvic cavity. This measured approximately 8 in. in length by 4in. in width. Small abscesses over the external wall of the uterus could be palpated and varied in size from that of a pea to a walnut. The ovaries were also enlarged and thought by rectal palpation to contain abscesses. The large abscess was evacuated on October 13, 1921. From this $\frac{1}{2}$ liter of thick creamy pus, streaked with blood,

was obtained. On October 23, 1920, the abscess was again evacuated. The pus this time was similar, but brown in color and exhibited an offensive odor.. Cultures were prepared from the purulent exudate, from which the following organisms were isolated: (a) B. pyogenes (Lucet), (b) streptococcus anginosus (Beta type). At this examination another abscess over the left horn and extending to the ovary was palpated. This was not opened. The animal became emaciated, showed slight intermittent fever, and loss of appetite. The lesions became progressively worse and slaughter was advised December 17, 1920.

## Necropsy

1. Gross Description. The cervix and corpus uteri are drawn downward to the left due to the adhesions and weight of the abscesses. The cervix appears normal. The body of the uterus is 3 cm. in length and its mucosa appears normal. The right uterine horn and right ovary are drawn downward and backward. An abscess is present on the right horn, 9 cm., anterior to the corpus uteri. This involves the perimetrium. The abscess is 3 cm.,in diameter. The right horn is adherent in many places to the rectum and colon. The carunculae are dull white in color. They measure from 1 to 15 mm., across the surface and rise to a height of 3 to 5 mm., from the gland mucosa. The gland mucosa is inflamed. There is no accumulation of pus in this horn. The left uterine horn proceeds forward and outward over a large abscess, to which it is firmly adherent. It turns abruptly at a distance of 10 cm., anterior to the body of the uterus, the turn forming an angle of 45 degrees. Very few carunculae appear in the first portion of the horn and

become more numerous in the anterior half. Below the horn and adherent to it, there is a large abscess, which measures 14 cm., in length and 10 cm., in width. Smaller secondary abscesses are present on the surface of the larger one. The abscess wall is thick and fibrous and its contents are thick, pasty and malodorous. The abscess is adherent to the pelvic wall and colon. The right tuba uterina is normal. The left tuba uterina enters the large abscess at a distance of 4 cm., from the tip of the horn. As it enters the abscess it is lost to view. The right ovary measures 4 cm., in length, 3 cm., in width and 2 cm., in depth. It contains a corpus luteum measuring 2.5 cm., in width and 2 cm., in depth. The center is cystic. Ventral to the corpus luteum, in the ovarian stroma, there is a cyst, measuring 17 mm., in diameter. Many Graafian follicles appear on the surface of the organ. The left ovary is buried in the large abscess from which it cannot be differentiated.

2. Microscopical Descriptions. The cervix appears normal. The uterus shows a quite uniform degeneration of uterine glands. This change is especially marked in the glands nearer the lumen of the organ. The epithelium of the glands shows granular degeneration of the cytoplasm, with only remnants of cells remaining. The nuclei are markedly pyknotic. Some show a marked infiltration with lymphocytes. The epithelium of the mucosa is in most places desquamated. In the lumen of the organ, there is occasionally seen an exudate, consisting of inspissated serum, lymphocytes and large mononuclear cells. There is an increased activity on the part of the mucosa of the right uterine tube. Considerable mucus appears in the lumen, together with marked losses of epithelium. A few lymphocytes appear in the lumen which also infiltrate the mucosa.

The right ovary presents many Graafian follicles which show marked cystic changes. These range in size from very small follicles to those measuring 2 to 3 mm., in diameter. The nuclei of cells of the membrana granulosa are markedly pyknotic. The liquor folliculi in place of being very light, is precipitated as dense, homogenous, deep eosin staining material. An example of this is illustrated in figure 3. Sections of the wall of the large cyst show it to be composed of an edematous thick supporting connective tissue structure. The theca folliculi and membrana granulosa are flattened out through great distention of the follicle. The primitive follicles appear normal. In one section there is also present a small corpus luteum cyst. This is approximately 1 mm., in diameter. The central portion is composed of a deep eosin staining homogenous coagulated fluid. The inner border is composed of lutein cells, supported by connective tissue and remains of the theca folliculi. Corpora albicantia are numerous throughout the ovarian cortex. Sections of the wall of the large abscess, show the wall to be composed externally of dense collagen fibrils. Toward the inner wall the connective tissue becomes more fibrillar. This shows quite a uniform infiltration with polymorphonuclear leukocytes, large mononuclear leukocytes, and plasma cells. The inner portion is almost entirely composed of exudative cellular material, supported by young connective tissue fibers.

3. Bacteriological Findings:

(1) Body of uterus, sterile.

(2) Right cornu uteri, B. pyogenes (Lucet)

(3) Left cornu uteri, sterile.

(4) Right uterine tube, sterile.

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(5) <u>Right</u> <u>ovary</u>, <u>sterile</u>.

(6) <u>Abscesses</u>,   (a) <u>B</u>. <u>pyogenes</u> (Lucet)

               (b) <u>Streptococcus</u> <u>anginosus</u> (Beta type)

4. <u>Diagnosis</u> and <u>Discussion</u>.

    1. Mild non-suppurative metritis.

    2. Catarrhal inflammation of right uterine tube.

    3. Cystic degenerative of Graafian follicles and small corpus luteum cyst in right ovary.

    4. Peri and para uterine abscesses, with abscess formation about the left tuba uterina and left ovary.

The persistence of infection about the uterus without involvement of that organ, to any marked extent is remarkable. The presence of large periuterine abscesses would undoubtedly lead to permanent sterility through adhesions, even though the genital organs themselves remain comparatively free from infection. The changes in the Graafian follicles may be the result of inflammation on disturbed uterine function. This is not known.

## Case 4.

<u>Clinical</u> <u>History</u>. Pauline, Holstein cow, age 2 years. Last pregnancy terminated September 6, 1920, with a premature delivery of the fetus. The fetus was coated with a yellowish brown, semi-turbid, tenacious exudate. The placenta was retained and removed 24 hours following parturition. Rectal examination and palpation of genital organs on October 23d, revealed a large abscess, the size of an orange in the left ovary. This was evacuated through the vagina. The cow suffered considerable shock following the operation, lactation became diminished for a number of hours. Had symptoms of severe abdominal pains, and loss of appetite for 12 hours. Gradually

returned to normal. Examined again on November 2d, the abscess had again enlarged with marked adhesions between left ovary and the uterine horns. The abscess became larger very rapidly with development of marked adhesions throughout the pelvis. Animal was regarded as incurably sterile and advised owner to slaughter. Cultural studies of the purulent exudate were made from time to time. The bacterial flora consisted of (a) B. pyogenes (Lucet) (b) Staphylococcus candidus (Cohn) and (c) Streptococcus fecalis, (Alpha type). The animal was slaughtered on December 17, 1920.

## Necropsy

1. Gross Description. Carcass is poorly nourished. There is an abscess 28 by 23 cm., situated in the left pelvic cavity. This is adherent to adjacent muscles, broad ligament, uterus, left ovary, intestines and peritoneum. It is well encapsulated and easily dissected from its attachments. The walls are thick and within them, there is contained a large quantity of pus. The pus is partly caseous and partly liquified. The odor is extremely offensive. The vagina is normal. The cervical canal of the uterus also appears normal. The right cornu uteri runs in a normal direction forward, downward and outward, and the tip of the horn is curved backward to the corpus uteri where the ovary is attached. The right horn and body of the uterus contain no pus. The carunculae are very prominent. The tips are deeply congested, and measure 13 by 8 mm., across the surface. They rise from the gland mucosa on the average 4mm. The left uterine horn proceeds in an irregular manner forward and outward. It then turns upward and again downward, over a large parauterine abscess. It coils itself about this abscess and adheres

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closely to it. The tip of the cornu is pressed between the two abscesses and is lost to view in the inflammatory overgrowths. There is no pus in the lumen. The carunculae are large and appear inflamed. They average in size, across their flattened surface, 6 by 12 mm., and extend upward from the surrounding mucosa 2 to 3 mm. The right tuba uterina is normal in appearance. The left is not visible because of extensive abscess formation about it. The right ovary appears normal. It measures 3 cm., in length, 1.5 cm., in width and 1 cm., in depth. The left ovary is buried in the abscesses which have formed about it so that it is completely lost to view. The abscesses on the left horn, not included in the parauterine abscess previously described, are two in number. One lies beneath the horn, and the other anterior to it. The former is 12 by 15 cm., in size, and the latter 6 by 8. These abscesses and both horns are adherent to the pelvic walls, colon and rectum.

2. Microscopical Descriptions. The cervix is normal. The mucosa of the uterus is in the resting stage. The glands, for the most part, are very small. There appears to be in some areas, an over-growth of connective tissue, and in other areas the walls are edematous. There is an increase of lymphocytes, mononuclear leukocytes and plasma cells, in the stroma of the gland mucosa. In the acini of some of the glands, lymphocytes are present. A few peripheral glands show degeneration of epithelium. The right uterine tube is practically normal. In the right ovary there are many degenerating Graafian follicles. The cells of the membrana granulosa are partly degenerated, the nuclei of which show pyknosis and karyorrhexis. The liquor folliculi is replaced by a more heavily precipitated fluid. It appears homogeneous and is deeply stained

with eosin.  In some follicles where ova appear, the cells of the

cumulus oophorus are degenerated similar to those of the membrana

granulosa.  In the ovum the cytoplasm appears to have undergone a

hyaline degenerative change.  Other areas show small cysts, lined by

lutein cells.  These are small cystic remains of corpora lutea.

Corpora albicatia are numerous.

    3. Bacteriological Findings.

        (1) Corpus uteri, sterile.

        (2) Cornua uteri, sterile.

        (3) Right tuba uterina, sterile.

        (4) Right ovary, sterile.

        (5) Parauterine abscess, (a) B. pyogenes.

                                (b) Streptococcus fecalis(Alpha
                                                      type)

                                (c) B. proteus.

                                (d) Staphylococcus candidus (Cohn)

        (6) Periuterine abscesses, same except no B. proteus
            isolated.

    4. Diagnosis and Discussion:

        (1) Mild non-purulent metritis.

        (2) Cystic degeneration of Graafian follicles of right
            ovary.
        (3) Periuterine and parauterine abscesses.

The absence of bacteria in the genital organs cultured, is

comparable to the results obtained in case 3.  The infective process

seems to be completely localized with ability of the mucosa of the

genital tract to withstand the invasion of the organisms.  This

immunity from attack, no doubt, would not be permanent, for sooner

or later the progress of the infection would lead to definite

changes in the uterus and right uterine tube, such as have already

occurred in the left tuba uterina and left ovary.  The cystic

degeneration of medium sized Graafian follicles whether of importance or not, is at least an interesting observation.

## Case 5.

Clinical History. Aged Holstein cow. Lady Astrea. Calved normally in 1919, aborted April 15, 1920. Retained placenta following which she developed a severe metritis. She was three months recovering from this. She had no treatment after the placenta was removed. Examined 10/13/20, and pronounced incurably sterile. Slaughtered 10/29/20.

### Necropsy

1. Gross Description. The carcass is well nourished. The cervical canal is tightly closed, but does not appear inflamed. The body of the uterus and horns are thickened. The mucosa of the uterus is not inflamed. The carunculae are normal. The right uterine tube is adherent to the right horn of the uterus. The uterine tube is enlarged especially pronounced at the fimbriated end, and near the uterine end it appears normal. The tube is very tortuous at the fimbriated end. It is dilated to 1 cm., in diameter, and contains a clear straw-colored fluid. The lumen is crossed by interlacing bands giving it a multilocular appearance. The tube winds its course through a mass of connective tissue, which in some places is so dense that it cannot be definitely seen. Near the uterine end the tube is 2 mm., in diameter. At the fimbriated extremity, there appears an abscess, adherent to the uterine tube and right ovary. It measures 2 cm., in diameter, and is completely encapsulated within a thin wall. The pus it contains is not

malodorous, is pasty in consistency, and greenish yellow in color.
The left ovary is 4 cm., in length and 2.5 cm., in width. On the
surface there are many small follicles, and buried in the substance
of the ovary, there is a large corpus luteum. It is 1.5 cm., in
diameter at the surface of the ovary, and extends into its substance
13 mm. The right ovary is approximately 3.5 cm., in length and
2.5 cm., in width. It is encapsulated by a firm connective tissue
capsule. The ovary is very hard and the cut surface shows no
visible ripe follicles, except at one border, where a small portion
of normal appearing ovarian tissue appears. The intestines and
mesentery are firmly adherent to the right ovary.

2. Microscopical Description. The cervix is normal. The uterus
is normal. The uterine mucosa is in the resting stage, as shown
by the small inactive glands. The left tuba uterina is normal.
The right tuba uterina is normal at the isthmus. Sections across the
tube near the ampulla show a tube with a greatly thickened wall, due
to proliferation of connective tissue. There is also fibrous
thickening of the mucous folds. This has formed adhesions between
the folds leaving gland like structures enclosed within masses of
connective tissue. Those structures are lined with a low cuboidal
epithelium and contain precipitate of serous fluid and mucin. Some
have become greatly dilated by the retained fluid. In the center the
fibrosis has been so complete that the attachments extend across the
lumen and connect with similar areas on the opposite side. Some of
the mucosa folds do not appear to have undergone much thickening,
but in place are elongated and flattened out. Throughout the areas
of fibrosis, and in the lumen of the dilated structures, there are
lymphocytes and occasionally polymorphonuclear leukocytes. The
inflammatory cells are, however, not numerous. The left ovary shows

many small cystic Graafian follicles. The membrana granulosa is mostly degenerated, and the liquor folliculi is replaced by a thick, dark staining, homogeneous material. Some of the follicles show another type of degeneration, as pictured in figures 1 and 2. These show a greatly thickened theca folliculi and proliferated epithelial cells of the membrana granulosa and may be interpreted as atretic follicles. Some sections show small cystic corpora lutea. Surrounding the wall one sees connective tissue, lying within which is an area resembling the corpus albicans. The innermost portion of the wall is composed of a thin layer of lutein cells. The content of the cyst is not rich in albumin or mucin, for the precipitate is light and granular. The right ovary shows similar cystic changes of the Graafian follicles. The most marked lesion, however, is the marked increase of connective tissue, leaving a sclerotic organ. Sections through the abscess show a dense capsule of connective tissue. Toward innermost portion the cells of the exudate are closely packed. Polymorphonuclear leukocytes predominate, with plasma cells and large clear mononuclear leukocytes rather numerous.

   3. Bacteriological Findings.

       (1) Corpus uteri, sterile.

       (2) Right cornu uteri, B. pyogenes (Lucet).

       (3) Right tuba uterina, (a) Staphylococcus candidus (Cohn).

                              (b) Streptococcus ignavus. (Alpha
                                                          type)
                              (c) B. pyogenes (Lucet)

       (4) Right ovary, sterile. Cyst, B. pyogenes.

       (5) Left cornu uteri, sterile.

       (6) Left tuba uterina, sterile.

       (7) Left ovary, sterile.

(8) Tubal abscess. (a) B. pyogenes (Lucet).

(b) Streptococcus ignavus.

4. Diagnosis and Discussion.

(1) Hydrosalpinx of right tuba uterina.

(2) Abscess of fimbria of right tuba uterina.

(3) Sclerosis of right ovary, with cystic degeneration of of many Graafian follicles.

(4) Cystic degeneration of Graafian follicles of left ovary.

The fact that sterility existed in this case with only obstruction of the uterine tube on the right side indicates the close relationship between ovary and sterility. Evidently the ova in the left ovary were degenerated when set free, or the pathologic condition of the right side prevented fertilization and implantation of the ova from the left ovary.

## Case 6.

Clinical History. Holstein cow, age 12 years. Mabel Homestead. Calved normally Dec. 7, 1919. Bred Jan. 20, 1920; April 7, 1920; July 17, 1920. No history of disease. Examined Oct. 13, 1920. The examination showed hydrosalpinx in both uterine tubes, and both ovaries large and cystic. The animal was pronounced incurably sterile and slaughtered 10/29/20.

### Necropsy

1. Gross Description. The carcass is well nourished. The cervix is partially dilated. It appears normal in structure, except for small cysts which appear deep in the structure of the internal os uteri. A small tumor projects from the outer surface of the body of the uterus. It is 2 cm., in diameter and is located in the muscular

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coat of the uterus, and gives the rounded prominence seen on the serosa of the corpus uteri. The growth is soft, circumscribed, and pink in color. The mucosa of the <u>uterus</u> appears normal. The <u>right</u> <u>uterine tube</u> begins as a normal structure. It gradually enlarges until it reaches a diameter of 1.5 cm. The tube is hard, and runs a markedly tortuous course. It contains a thin straw-colored fluid in its lumen. The lumen is for the most part obliterated and replaced by interlacing strands of tissue giving it a multilocular appearance. At the fibria the tube is adherent to the right ovary. The <u>left</u> <u>tuba uterina</u> presents the same picture as the right, except that it is smaller and not adherent to the ovary at the fimbriated extremity. The <u>right</u> <u>ovary</u> is 3 cm., in length and 2 cm., in width. It is hard and fibrous in appearance, and is covered by a firm, hard capsule. In the center of the ovary there is a large persistent corpus luteum. It measures 1.5 cm., in diameter. The outer wall is yellow in color (2 mm., thick), and the center presents a hard dark red mass which is not adherent to the lutein tissue of the wall. The central mass is from $\frac{1}{2}$ to 1 cm., in diameter. The <u>left</u> <u>ovary</u> is comparable in size to the right one. It contains a corpus luteum and appears normal.

2. <u>Microscopical</u> <u>Description.</u> The <u>cervix</u> shows a normal mucosa. The internal portion adjacent to the corpus uteri, has many cystic glands deep in its wall, surrounded by the myometrium. The largest of the glands is 1.5 cm., in length and 1.5 cm., in width. The cystic glands are lined by simple cuboidal epithelium, and the outline is very irregular. The precipitated content of these cysts, is in most instances very small in amount, but in one it is dense, homogenous and eosin staining. The epithelium of the <u>uterine</u>

mucosa in some places, appears thicker than normal. This is evident in both cornua and in the corpus uteri. The epithelial cells are flat in place of columnar, in these areas, and are several layers in thickness in place of simple. Adjacent to these apparently squamous cell and thickened epithelial areas, the cells are simple columnar in type. This may be interpreted as "ectodermoisering", described by wall. The uterus appears normal in every other respect. Sections through the tumor, described in the wall of the corpus uteri, show it to be composed of smooth muscle, well circumscribed by connective tissue. The gland mucosa is in a resting state.. Sections through the enlarged tuba uterina show a comparatively thin wall, consisting almost entirely of connective tissue. The mucous membrane consists of a greatly flattened simple cuboidal epithelium. The folds of the mucosa are stretched and converge toward the center where the connective tissue is much more pronounced giving the central convergent folds great thickness. In this, collapsed adherent folds are completely surrounded by edematous connective tissue and appear as acini of glands. The adherent folds give the tube the appearance of multilocular cysts, all lined by the same type of low epithelium. They contain a faintly hematoxylin staining material indicating its mucous composition. Inflammatory cells are not numerous and when present consist of lymphocytes. The ovaries are sclerotic. Primitive Graafian follicles are very few in number, and corpora albicantia are very numerous. A few Graafian follicles show degenerative changes characterized by flattening and stretching of the membrana granulosa to a single layer of cells and replacement of the liquor folliculi by a more dense material having the staining reaction of mucin.

3. <u>Bacteriologic Findings</u>.

    (1) <u>Os uteri externum</u>. (a) <u>Staphylococcus aureus</u> (Rosenbach)

                    (b) <u>Staphylococcus aurantiacus</u>

                                        (Schröter)

                    (c) <u>B</u>. <u>coli</u> <u>communis</u>

    (2) <u>Corpus uteri</u>. Same as os uteri externum.

    (3) <u>Right cornu uteri</u>, <u>sterile</u>.

    (4) <u>Left cornu uteri</u>, <u>sterile</u>.

    (5) <u>Right</u> and <u>left tuba uterina</u>, sterile.

    (6) <u>Left</u> and <u>right ovaries</u>, <u>sterile</u>.

4. <u>Diagnosis</u> and <u>Discussion</u>.

    (1) Cystic uterine glands in wall of os uteri internum.

    (2) Leiomyoma in body of uterus.

    (3) "Ectodermoisering" of uterine epithelium (Replacement of simple columnar by stratified ectodermal, in some areas).

    (4) Hydrosalpinx, both uterine tubes.

    (5) Sclerotic ovaries, with cystic degeneration of most medium sized and large Graafian follicles.

Sterility in this case would have been absolute with only the presence of bilateral hydrosalpinx. In the study of the case, even though clinical history is not complete, in all probability a puerperal infection followed the last parturition. This then may be theoretically considered as the starting point of the infection which left in its wake, the irreparable pathologic changes in the uterine tubes. The cystic changes in the ovaries may or may not have significance. The leiomyoma was not sufficiently large to cause sterility had other portions of the genital tract been normal.

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## Case 7

Clinical History. Aged Holstein cow. Last parturition about
Sept. 1918. Suffered retained placenta. Came to our attention
Oct. 19, 1920. Diagnosed chronic cervicitis with hypertentrophy of
external os uteri. Deep-seated corpus luteum in right ovary.
Cultures prepared at this time from cervix obtained from sterile
swabs showed the following flora: (a) B. pyogenes, (b) B. coli,
(c) Staphylococcus aureus, (d) Staphylococcus aurantiacus. Corpus
luteum removed from right ovary and cervix painted with Lugol's
solution 10/30/20, again 11/10/20. and 12/18/20. On Jan. 10, 1921,
removed a large portion of hypertrophied cervical folds, at which
time also, a quantity of pus was located in the uterus. The left
horn was hard upon palpation. Adhesions were also noted between
ovaries and uterus at this examination. Examined again Feb. 28,
1921, which revealed a continued marked cervicitis and purulent
metritis. The right ovary was the size of a lemon, and firm upon
rectal palpation. The animal was pronounced incurably sterile and
slaughtered March 5, 1921.

## Necropsy

1. Gross Description. The carcass is poorly nourished. There
is a large quantity of mucopurulent discharge in the vagina. The
walls of the vagina are inflamed. There are two cysts in the floor
of the vagina (2 cm., in diameter). These contain a thick mucinous
material. The folds of external os uteri are hypertrophied.
Granular, pin point in size, inflammatory areas stud the mucous
surface. The cervical canal contains much thick, yellow pus. The
mucosa of the body of the uterus is rather dry. The right horn
of the uterus is hard and indurated and averages 1/3 smaller than

the left one. At the bifarcation of the uterus situated above and to the right, there is an adhesive mass of tissue, adherent to the serosa of the horn. At this portion the wall is hard and thick. This horn contains considerable quantity of white pus, which is creamy in consistency. The wall of the right horn is 1.5 cm., in thickness. The <u>left</u> <u>uterine</u> <u>horn</u> is flatulent and indurated. The wall is 1.5 cm., in thickness. It contains pus as does the right side of the uterus. The <u>right</u> <u>uterine</u> <u>tube</u> is normal at the isthmus. At a distance of 2 cm., from the uterus it becomes very tortuous. It gradually enlarges until it reaches 1 cm., in diameter. It is distended with a clear serous fluid. The fimbriated end is obliterat-ed by a mass of connective tissue. This mass is 10 cm., in width, 4 cm., in depth and 7 cm., in length. It is covered by mesenteric fat which adhers to it and brings a loop of the small intestine in close apposition. In the center of this connective tissue mass there is considerable pus. The connective tissue first described forms the wall of the abscess. This wall is adherent to the right ovary on one side, and to the serosa of the uterus on the other side. The <u>abscess</u> extends into the <u>right</u> <u>ovary</u> and is responsible for its large size. The pus in the <u>ovarian</u> <u>abscess</u> is greenish, thick and pasty, and has a nut-like odor. The left <u>tuba</u> <u>uterina</u> is enlarged, and the walls are thick and hard. The right ovary is covered by dense connective tissue being almost completely buried in the mass. It measures 7 x 3 cm. It contains an old corpus luteum 2 cm., in diameter. The ovary appears sclerotic. The <u>left</u> <u>ovary</u> is normal. It measures 5 x 3 x 1.5 cm. It contains a corpus luteum 1.5 cm., in diameter. Many small Graafian follicles are also present.

    <u>Microscopical</u> <u>Description</u>. The epithelial lining of the vagina is stratified ectodermal. The outer portion shows some destruction

of epithelial cells, but not pronounced. The inflammatory exudate
seen grossly in the vagina is evidently drainage material from the
uterus. Lying on the epithelial surface, many polymorphonuclear
leukocytes are seen. The cyst described grossly is composed of a
lining of simple columnar epithelium, thrown more or less into folds.
The cavity contains a faint neutral staining homogeneous material
probably mucus. Throughout this material cholesterin clefts and
clefts of fatty acid crystals are numerous. Tiny globules of neutral
fat are also seen. The cervix uteri shows extensive superficial
proliferation. This has in many instances occluded the openings of
the cervical folds so that gland like structures without outlet to
the surface appear buried beneath this proliferated connective
tissue. The cells of these occluded folds contain much mucus, as
do also the epithelial cells of the surface mucosa. In the zone of
fibrosis, many small capillaries are seen, and quite uniformly
infiltrating the area are plasma cells and large mononuclear
leukocytes. The body of the uterus and the cornua show marked
changes in the mucosa. The lining epithelium is very irregular in
its state of preservation. In one area for a short distance, the
epithelium appears as stratified ectodermal six to eight cells in
thickness. It ends abruptly and is continued on each end as simple
columnar epithelium. The columnar epithelium contains much mucus
but the "ectodermoised" area is free of this secretion. Attached to
the epithelium and infiltrating it there remain occasionally poly-
morphonuclear leukocytes. In some places the epithelium is necrotic.
Beneath the epithelial surface the mucosa is densely infiltrated
with leukocytes. Plasma cells predominate with polymorphonuclear
leukocytes and lymphocytes also numerous. They diminish in number
in the deeper structures of the mucosa and young connective tissue

cells become more prominent. The uterine glands are markedly atrophied. In many places the inflammatory reaction has produced proliferative changes occluding the outlet of the glands. This has resulted in dilation with retention of mucus. These have a very irregular and tortuous outlines. The epithelium is low cuboidal and is not seriously damaged. Some glands show a greatly flattened epithelium surrounded by rather dense fibrillar connective tissue, and in the lumina of many, polymorphonuclear leukocytes are seen. Through-out the myometrium there are several collections of cellular infiltrations similar in character to those described for the endo-metrium. The mucosa near the tips of the horns is much less involv-ed. Many normal glands still remain but superficial changes have begun which suggest the gradual transition to the chronic changes as described for the other portion of the uterus. In the left tuba uterina the wall is thickened, due to an increase of connective tissue. The folds of the mucosa are greatly thickened, and many adhere to one another. In the central portion of the lumen, the folds converge where they have become adherent to folds on the opposite side of the tube. Due to this overgrowth, many folds have been brought together at their base, so that they appear as acini of glands below the connective tissue mass. All of the folds, even where adherent, are still lined by simple columnar epithelium, which contains much mucus. The connective tissue is infiltrated with lymphocytes, and in the lumina between adherent folds, polymorpho-nuclear leukocytes and lymphocytes are occasionally found. The right tuba uterina is normal at the isthmus. Above this the tube is large. The wall is much thicker and edematous. The folds of the mucosa are thickened and elongated. They converge toward the center of the lumen where they form a thick mass of edematous connective

tissue, lined by simple columnar epithelium. The other folds are likewise covered by the same type of epithelium. Considerable exudate is present in the lumen of the tube and between adherent folds. This consists of inspissated serum, fibrin and a few poly-morphonuclear leukocytes and lymphocytes. The ovaries show a marked lymphocytic infiltration, which in some areas forms dense masses. The larger follicles show cystic changes, marked only by increased density of liquor folliculi. The smaller follicles show quite uniformly degenerative changes in the ova, and cells of the membrana granulosa. These changes are not marked and are difficult to interpret.

    3. Bacteriological Findings.

        (1) The os uteri externum, and uterus.

            (a) B. pyogenes (Lucet).

            (b) Streptococcus non-hemolyticus II (Gamma type).

            (c) Staphylococcus aureus (Rosenbach).

        (2) Uterine tubes, Right tubo-ovarian abscess, and Right ovary.

            (a) B. pyogenes.

            (b) Streptococcus non-hemolyticus, II.

            (c) Staphylococcus epidermidus (Gordon).

        (3) Left ovary, Sterile.

    4. Diagnosis and Discussion.

        (1) Vaginitis.

        (2) Chronic purulent cervicitis and metritis.

        (3)Hydrosolpinx of both tubes.

        (4) Right tubo-ovarian abscess.

        (5) Right ovary sclerotic, cystic Graafian follicles.

        (6) Left ovary shows cystic Graafian follicles.

The presence again of cystic degenerative changes in the Graafian follicles is interesting. As a result of the long continued metritis chronic changes have occurred which have left the uterus in a state of irreparable destruction with loss of breeding efficiency. The causes of sterility are apparent.

## Case 8.

Clinical History. Holstein cow, age 4 years. Last calf born Feb. 1919, normal. Did not retain placenta. Cow has never aborted a calf. Owner had infectious abortion in herd 5 years ago. Cow has regular estrus periods. Animal was bred twelve times following birth of calf in Feb. 1919, and at no time did conception take place. Slaughtered as a tuberculosis reactor Dec. 1920. No evidence of tuberculosis found at post mortem examination.

## Necropsy

1. Gross Description. The vagina is normal except near the vulva where there is evidence of granular venereal disease. The cervical canal is open, one being able to insert the small finger without difficulty. The uterus appears normal on its serous surface. Both uterine tubes are normal. The left ovary is normal in size. A corpus luteum 2 cm., in diameter is imbedded in the ovarian substance. It is hemorrhagic, many small blood vessels being present about the periphery. Two large follicles 15 mm., in diameter are present on the surface. These appear to be cystic. The right ovary is normal in size. On the upper surface there appears a cyst 1 cm., in diameter. The walls are thick. A small scar (corpus albicans) appears on the border of the cyst.

2. <u>Microscopical</u> <u>Description</u>. The <u>vagina</u> is normal. The <u>cervix</u> is normal. In the <u>uterus</u> the superficial glands of the mucosa are active, appearing according to Marshall's descriptions as in the period of proestrum. These glands show degeneration and desquamation of the epithelium and increased cellular and mucus exudate in their lumina. The cells of the exudate are mostly lymphocytes and large mononuclear leukocytes. Erythocytes are not evident outside the blood vessels. The deeper glands are normal and in a state of rest. The <u>tubae</u> <u>uterinae</u> are normal. The <u>ovaries</u> appear for the most part normal, the primitive ovarian follicles are normal, as also are the larger ones that are present in the several sections taken.

3. <u>Bacteriological</u> <u>Findings.</u>

    (1) <u>Os</u> uteri <u>externum</u>, <u>corpus</u> <u>uteri</u>, and <u>right</u> <u>horn</u>.

        (a) <u>Staphylococcus</u> <u>aureus</u> (Rosenbach)

        (b) <u>Staphylococcus</u> <u>aurantiacus</u> (Schröter)

        (c) <u>Staphylococcus</u> <u>candidus</u> (Cohn)

    (2) The <u>left</u> <u>uterine</u> <u>horn,</u> <u>uterine</u> <u>tubes</u> and <u>ovaries</u> are <u>sterile</u>.

4. <u>Diagnosis</u> and <u>Discussion.</u>

In this case the only suggestions of pathological changes are obtained from study of the endometrium. The changes seen do not, however, seem commensurate with absolute sterility. Possibly altered secretion, which cannot be judged histologically, played an important role. Nutritional disturbances may also have been an exciting factor. Any hypothesis proposed can have no scientific support in the light of present knowledge. Cases such as these are difficult to account for. The possibility of the corpus luteum of the left ovary influencing conception, must also be taken into account, the enucleation of which might have cleared up the

condition.

## Case 9

Clinical History. Aged Hereford cow. Several calves were born normally after which the animal failed to conceive. Had been sterile one year when brought to our attention. Clinical examination reveal- ed nothing but cervicitis. This condition was treated regularly with Lugol's solution for two or three months. Corpora lutea were enucleated from time to time. Slaughtered because of sterility on Dec. 15, 1920..

## Necropsy

Gross Description. The carcass is well nourished. The external os uteri shows many hypertrophied folds. The cervical canal contains a thick tenacious discharge. This is transparent and glistening. In some places it is streaked with blood. In the wall of the corpus uteri there is a tumor 13 mm., in diameter. It is rounded and well circumscribed. The mucosa of the uterus is normal. The walls are 8 mm., in thickness. The right tuba uterina appears normal. It contains a white colored tenacious mucus. The left uterine tube is normal except at the fimbriated end, where it is adherent to the left ovary. The tube here is slightly dilated as in hydrosapingitis. The right ovary contains many Graafian follicles. These in place of containing a clear serous fluid, contain a mucinous one. The ovary is normal in size. The left ovary is normal in size. There is a corpus luteum embedded in the ovarian substance. The corpus luteum is surrounded by a tough capsule, except in a small area at the surface where a small portion of the yellow body protrudes from the surface. The corpus luteum is 2 cm., in irregular diameter. In the center of the yellow body, there is a cystic cavity, containing a

clear fluid. This cavity is 4 mm., in diameter. Over the remainder of the surface of the ovary, there are many Graafian follicles. A corpus luteum lies on the broad ligament. It measures 1.5 cm., in diameter and is very hard in consistency. This represents a recently enucleated corpus luteum which is undergoing absorption in the peritoneal cavity.

2. Microscopical Description. The cervix is normal. The uterus is practically normal. A few glands of the mucosa show dilation and a flattened epithelium surrounded by rather dense circular bands of connective tissue. In others and surrounding some, lymphocytes are seen. The glands are in a beginning state of activity. The tumor described in the wall of the corpus uteri is composed almost entirely of smooth muscle. It is well encapsulated. The left tuba uterina is normal except at the fimbriated end, where it is dilated and adherent to the ovary. The folds of the mucosa are adherent near their tips and the spaces formed between them are lined with low cuboidal epithelium. The folds are greatly stretched and thin. This gives the tube the appearance of small multiple cystic cavities. There is a precipitate in the lumina, between the folds, indicating the previous presence of a serous exudate. Only occasional collections of lymphocytes are seen in the exudate. Figure 4 illustrates this condition. The right tuba uterina is normal. The left ovary contains many corpora albicantia. The medium sized follicles are cystic, as indicated by the replacement of the normal liquor folliculi by a fluid precipitating much albumin and containing much material staining like mucin. Other follicles show atretic degeneration. Primitive Graafian follicles are very few in number, and the organ appears to be sclerotic. Sections obtained from the

wall of the small cyst of the corpus luteum show it to be composed
internally of a flattened layer of lutein cells, supported by a
loose connective tissue framework. This is derived from ingrowths of
connective tissue into the corpus luteum from the supporting
membrane of the Graafian follicle. Beneath this the solid cords of
lutein cells, with rich blood supply, are seen. The central cavity
contains a small amount of debris; precipitate of the fluid it
contained. Sections of the enucleated corpus luteum, found in the
peritoneal cavity, show a uniform necrosis of all lutein cells.
These appear as granular eosin staining areas without definition.
Small clear spaces left by neutral fat are abundant. The right
ovary shows cystic and degenerated follicles as described for the
left one. It also appears somewhat sclerotic. Some of the ovarian
arterioles show hyaline degeneration of the media.

    3. Bacteriological Findings.

        (1) Os uteri externum, Staphylococcus epidermidis (Gordon)

        (2) Right cornu uteri, Streptococcus mitis (Alpha type)

        (3) Corpus uteri, left cornu uteri, uterine tubes and
            ovaries are sterile.

    4. Diagnosis and Discussion.

        (1) Leiomyoma of corpus uteri.

        (2) Hydrosalpinx of abdominal osteum of tube with
adhesions to right ovary.

        (3) Sclerotic changes in ovaries, and cystic degeneration
of follicles.

        (4) Persistent corpus luteum in left ovary, with central
cystic degeneration.

Sterility in this case can only be accounted for histologically by the hydrosalpinx in the right tuba uterina, sclerotic changes in ovaries, and cystic degeneration of ovarian follicles. The persistent corpus luteum which formed so frequently in the left ovary was also a contributing factor. The changes seen indicate a one time virulent infection, leaving only as a result, the changes in the right tuba uterina, and possibly the altered ovarian function. The case exemplifies further the close relationship that exists between the ovaries and sterility, involvement usually being bilateral.

## Case 10

Clinical History. Holstein cow. Age about four years. Presented at clinic for examination July 24, 1920. Past breeding unknown. The vagina was inflamed, and the walls were adherent. This created a blind ending, just anterior to the urethral opening. The right ovary was found to be cystic. The cyst was approximately the size of an English walnut. This cyst was ruptured several times. The right uterine tube was enlarged, and thought to be hydrosalpingitis. In the left ovary a corpus luteum was palpated. This was enucleated on September 1, 1920. It was impossible to dislodge it before this date due to deep embedding. Animal appeared in estrum Sept. 3, 1920, but was not bred because of the occluded vagina. Was condemned as incurably sterile and slaughtered for food purposes Dec. 17, 1920.

## Necropsy

1. Gross Description. Carcass exceptionally well nourished. The vagina is closed just anterior to the urethral orifice. The

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stricture appears to be the result of an inflammatory reaction which brought the vaginal walls in close apposition and united them by scar tissue. The center of the stricture presents a small rounded opening which communicates with the anterior portion of the vagina. The opening is 3 mm., in diameter. Anterior to the stricture the vagina has narrowed to 1.5 cm., in width. The cervix appears normal. The vagina enlarges at the external os uteri so that this structure is easily accommodated. The body of the uterus is normal. The left horn of the uterus is 3 cm., in cross diameter, at a point just anterior to the body. The right uterine horn is 5 cm., in diameter in the respective area, and continues to its extremity to be approximately twice as large as the left one. The right horn is flacid. The uterine mucosa appears normal. The right tuba uterina is normal at the isthmus. It gradually enlarges until it reaches a diameter of 8 mm., at the fimbriated extremity. This enlarged area adheres to the right ovary. It contains a clear, watery, straw-colored fluid. The lumen is crossed by strands of the mucosa which are adherent to one another, giving the lumen a multilocular appearance. The left tuba uterina is normal. The right ovary measures 5 cm., in length, 4 cm., in width and 3 cm., in depth. It appears cystic. A large cyst about 2 cm., in diameter is present. It contains a clear straw-colored fluid, which coagulates upon leaving the cyst. The wall of the cyst is 4 mm., in thickness. The inner border is yellow, while the outer portion appears white. The yellow portion is 2 mm., in thickness. This represents the remains of lutein cells, from a corpus luteum which passed through cystic degeneration in place of normal absorption. (This corpus luteum cyst was broken per rectum several times, and each time

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reformed). The left ovary is normal in size. It contains on its surface 3 large Graafian follicles 6 mm., in diameter. There is also a corpus albicans on its surface measuring 2 mm.,in diameter.

2. Microscopical Description. Over the mucosa of the vagina and adherent to it, there is considerable inflammatory exudate consisting of desquamated epithelial cells, large mononuclear leuko-cytes, and lymphocytes and fibrin. The epithelium is intact in most places, only occasionally appearing irregularly exfoliated. Beneath the epithelium there is a uniform distribution of lymphocytes and plasma cells, and occasional normal lymph follicles. The remainder of the wall appears normal. The cervix appears normal. The corpus uteri shows an extensive atrophy of mucous glands and a dense fibrous tissue replacement. Only occasionally are glands seen. Lymphocytes infiltrate the mucosa quite uniformly, more numerous than normal. In the cornua the epithelium is in many places necrotic and in others entirely lost. The glands of the mucosa show degeneration as evidenced by loss of epithelium, and lymphocytic infiltration into the lumina. This may correspond to proestrum as described by Marshall, but the destruction seems too extensive not to be considered pathological. The stroma of the endometrium is edematous. There does not appear atrophy of glands, but simply degenerative changes. In the lumen of the cornua, there is con-siderable cellular debris, consisting of desquamated epithelial cells, lymphocytes and mononuclear leukocytes. The left tuba uterina is normal. The right tuba uterina is normal at the isthmus. At the ampulla it becomes dilated and corresponds histopathologically to the descriptions previously given for hydrosalpinx. The walls are thickened to a great extent. The mucous folds do not cross the

lumen, but are all flattened out and incorporated in the wall, with entire loss of epithelium. A few normal appearing folds remain. The enlarged lumen is lined by a simple flat epithelium (stretched columnar epithelium). The right ovary contains many corpora albicantia. Some follicles show degenerative changes. In one the membrana granulosa and the theca folliculi have greatly atrophied, only remnants of these layers remaining. The ovum is caught in and surrounded by proliferations of connective tissue, which extend along the side formerly occupied by the cumulus oöphorus. In this area lymphocytes are numerous. Many follicles appear normal, while some medium sized ones appear cystic. These contained a heavy homogeneous eosin staining precipitate, in place of the normal liquor folliculi, and the membrana granulosa is flattened out. Small primitive follicles are not numerous but appear normal. The wall of the cystic corpus luteum is lined by an edematous connective tissue, beneath which a thick layer of lutein cells are seen, supported by dense connective tissue. The left ovary is practically normal.

3. Bacteriological Findings.

    (1) Os uteri externum, corpus uteri, left cornu uteri, uterine tubes, and ovaries are sterile.

    (2) Right cornu uteri, Staphylococcus epidermidis (Gordon)

4. Diagnosis and Discussion.

    (1) Stricture of vagina resulting from vaginitis.

    (2) Chronic proliferative changes in uterus with atrophy of uterine glands.

    (3) Hydrosalpinx of right tuba uterina.

    (4) Many atretic and cystic degenerated Graafian follicles in right ovary.

(5) Cystic corpus luteum in right ovary.

The presence of staphylococci in the right cornu uteri cannot be regarded as responsible for the changes seen. Its presence can best be defined as a chance invader and not at the time of necropsy, playing a pathogenic role. Sterility in this case would have been absolute with only the presence of vaginal stricture, so great were the bands of attachment. The uterine changes and hydrosalpinx are, as is the vaginal change too, the end result of chronic infection produced most likely by streptococci or B. pyogenes or both. The degenerative changes described for the right ovary are interesting, but possibly not an important contribution.

## Case 11.

Clinical History. Scrub cow, about $3\frac{1}{2}$ years of age. Animal was used as a control in the abortion herd at University Farm. Purchased from South St. Paul May 20, 1918. Age at that time 8 months. Agglutination test for infectious abortion in June 1918, positive at 1-20, and slight at 1-50. September 1, 1918, test positive only at 1-20. Bred December 30, 1918. Pronounced pregnant February 15, 1919. Examined for pregnancy April 7, 1919. Found not pregnant. The animal was seen to have considerable blood on rear quarter two weeks previously, and was considered to have aborted. Large corpus luteum in right ovary was enucleated. Bred June 2, 1919. Pronounced pregnant October 23, 1919. Agglutination test for infectious abortion on November 22, 1919, was negative. A calf was born normally March 4, 1920. Agglutination test March 9, 1920, negative. Bred May 10, 1920. Pronounced pregnant August 11, 1920. Agglutination test August 13, 1920, negative. A calf was born normally February 15, 1921. Agglutination test on calf and dam was

was negative February 15, 1921. The calf died February 18, 1921. The cow developed a discharge from the uterus after parturition. This continued until she was slaughtered on March 4, 1921. The animal was condemned as a tuberculosis reactor and as a metritis had followed the last parturition, opportunity was taken for pathological and bacteriological study.

## Necropsy

1. Gross Description. The carcass is well nourished. The mucosa of the vagina is slightly inflamed. The vagina contains a large quantity of thick, viscid mucous discharge, which is streaked with pus. The cervical canal is dilated and contains much discharge similar to that seen in the vagina. Otherwise it appears normal. The os uteri internum is inflamed. The body of the uterus is deeply congested. The horns of the uterus are deeply congested and each contains thick mucus, which in some places is streaked with pus. The pus is thick and yellow. The carunculae are large and prominent. The surface of the carunculae range from ½ to 2 cm., in diameter and they rise from the gland mucosae from 3 to 6 mm. The uterine tubes are normal. The ovaries are small, but appear normal.

2. Microscopical Description. The cervix is normal. The uterus shows considerable exudate in the lumen, lying on the epithelial surface. The exudate consists of inspissated serum, mucus, polymorphonuclear leukocytes and lymphocytes. Many desquamated epithelial cells are also present in the exudate. In the epithelium there are many polymorphonuclear leukocytes. The epithelial cells contain much mucus. The epithelium is simple columnar in type. Beneath the epithelium collections of leukocytes are numerous.

These consist for the most part of polymorphonuclear, but lymphocytes are also numerous. The uterine glands are normal and in a state of rest. Occasional leukocytes are seen in lumina of these glands. The blood vessels are engorged with blood. The <u>uterine tubes</u> are in a state of congestion. Some hemorrhage into the lumen exists. The <u>right ovary</u> is normal. Primitive follicles and medium-sized follicles are numerous and appear normal in structure. In the <u>left ovary</u>, in the sections studied, several follicles appear in a state of atretic degeneration. Others show a changed liquor folliculi, the coagulum being dense, homogeneous and eosin staining. Other structures of these follicles are normal. Very few normal Graafian follicles appear in the sections studied.

3. <u>Bacteriological Findings</u>.

    (1) <u>Uterus</u>  (a) Streptococcus viridans (Alpha type)

            (b) Staphylococcus epidermidis (Gordon)

            (c) Staphylococcus aureus (Rosenbach)

            (d) Sarcina lutea

            (e) Torula rosea

    (2) <u>Uterine tubes, sterile.</u>

    (3) <u>Ovaries</u>, sterile.

4. <u>Diagnosis</u> and <u>Discussion</u>.

This case cannot be regarded as a case of sterility for the animal was not sterile. It was studied in this series as an illustration of mild puerperal metritis. The left ovary shows cystic degeneration of many ovarian follicles, others being atretic. It is not known of what significance such change may be.

## Case 12.

Clinical History. Holstein cow. Age 7 years. Cow gave birth to a calf as a 2 year old. Since that time she has been sterile. Had irregular periods of estrum and when bred did not conceive. Animal was condemned because of sterility and slaughtered for food March 1921.

### Necropsy

1. Gross Description. The carcass is well nourished. The vagina is normal. The cervix is normal. The body of the uterus is normal. In the left horn there is a very slight amount of muco-purulent discharge. The right horn is normal. The uterine tubes are normal. The left ovary is normal. The right ovary is normal. It contains a normal corpus luteum measuring 2.5 cm., in width and 1.5 cm., in depth.

2. Microscopical Description. The cervix is normal. The uterus shows a thickened epithelium, which occurs only in places, other portions adjacent to the thickened areas being normal. In the thickened areas the cells are flat and stratified to 6 or 8 layers in depth. This epithelium appears as stratified ectodermal and possibly corresponds to "ectodermoisering" described by Wall. The endometrium otherwise is normal, as also are the other uterine coats. The uterine tubes are normal. The ovaries are both equally involved. They present, in several sections studied, degenerative changes of the Graafian follicles. In one section a Graafian follicle is seen with the following changes: The liquor folliculi is replaced by a dense coagulum taking a neutral stain. The epithelial cells of the cumulus oöphorus are mostly degenerated, a few cells of the corona radiata, however, still remain. The zona

pellucida is dark in color, and the vitellus in place of being
granular and containing vacuoles is replaced by a dense homogeneous,
neutral staining substance. The nucleus is not present. The cells
of the stratum granulosum are in a state of degeneration. Many are
losely distributed about the follicle. Nuclei show degenerative
changes, for the most part pyknosis. Other Graafian follicles of
medium size show the same dense coagulum replacing the normal light
coagulum of the liquor folliculi, with stretching and flattening of
the stratum granulosum. The theca folliculi is greatly thickened.
Primitive ovarian follicles are numerous and appear normal. Corpora
albicantia are numerous.

    3. Bacteriological Findings.

        (1) Cultures from the cervix uteri, uterus, cornua uteri,
              uterine tubes and ovaries remained sterile.

    4. Diagnosis and Discussion.

        In this case, as in cases 8 and 9, the etiology of
sterility is not clear. The "ectodermoisering" described in the
uterus does not seem a sufficient change to cause absolute sterility
such as existed in this animal. The ovarian changes, cystic
degeneration and atretic degeneration, are the only histological
changes throwing any light upon the possible etiology. But it cannot
definitely be agreed that this change is present in all follicles.
All organs cultured were sterile. There may have been a mild
puerperal infection following the birth of the calf, at two years of
age. Certainly at the time of necropsy, no infection existed.

### Case 13.

.Clinical History. Holstein heifer, age 2 years. Showed normal
periods of estrum. Examination early in December 1920, to determine

inability to conceive, resulted in finding an occluded vagina. This was thought to be a case of arrested development of the genitalia. The animal was closely bred, but not a twin. Condemned for sterility and slaughtered for food purposes December 18, 1920.

## Necropsy

1. Gross Description. The vagina is 5 cm., in length. A short distance anterior to the urethral opening the walls of the vagina come together, forming an almost complete stricture. In the center of the stricture, an opening 2 mm., in diameter exists. Anterior to this, the vagina enlarges slightly. There is no cervix uteri. From the vagina proceeding anteriorly, there are two hard and fibrous tubes, contained within a common capsule. The length of these tubes, as they run parallel to one another, is 30 cm. They divide at this point and proceed forward at angles to one another. At a distance of 14 cm., from the dividing point, they end abruptly. The center of these tubes have lumina 4 mm., in diameter, which near the anterior extremity, dilates to 1 cm. The largest diameter of each tube is 1.5 cm. At the anterior end of each tube there is a maldeveloped uterine horn. The right horn is larger than the left one, and is nearly spherical. It measures 9 cm., in length and 10 cm., in cross diameter. The walls are very thin and the horn is distended with a yellow colored turbid fluid. The left horn is similar in appearance to the right one. Its measurements are 9 cm., in length and 5.5 cm., in cross diameter. The uterine tubes run backward from the tip of each horn, and end in an indistinct fimbria. The right ovary is slightly larger than the left. It measures 4.5 cm., in length and 2 cm., in width. Over the surface of the organ there are many small follicles. These in place of containing a clear watery liquor

folliculi, contain an opalescent semi-solid fluid, probably an expression of small Graafian follicle cysts. The <u>left</u> <u>ovary</u> is also cystic and contains a corpus luteum 3 cm., in depth and 2 cm., in width.

2. <u>Microscopical</u> <u>Description.</u> Sections through the remains of the Müllerian tubes show a lining of low cuboidal epithelium, in most places simple, in some areas stratified. Beneath the epithelial surface the connective tissue is dense and consists for the most part of collagenous fibrils. A few large simple tubular glands are occasionally seen. The inner surface of the tubes is thrown into low rounded folds. Smooth muscle is distributed throughout the connective tissue and is more abundant in the deeper structures. In the partially developed <u>uterus</u> the mucosa is lined throughout, with a thick stratified ectodermal epithelium. Beneath this there is a thick coat of connective tissue distributed evenly along the mucosa. This tissue is exceedingly edematous. The uterine glands lie beneath this coat of connective tissue. They are surrounded by dense connective tissue bands and the epithelium is low columnar. Some contain much mucus. The surface of the mucosa is thrown up into small irregular folds. There does not appear differentiation into gland mucosa and carunculae. The muscular coat is thin. The entire thickness of the mucosa does not exceed the ordinary development of the endometrium of a normal uterus. The <u>uterine</u> <u>tubes</u> appear normal in structure. In the <u>ovaries</u> medium sized Graafian follicles are exceedingly numerous. These show quite uniformly a theca folliculi of increased thickness, a quite normal appearing stratum granulosum, and a thick homogenous, neutral staining coagulum of the liquor folliculi. In the several sections studied, no ova are

present. Small primitive follicles are also few in number.

   3. Bacteriological Findings.

      (1) Cultures prepared from the right cornu uteri, left

          cornu uteri and ovaries are sterile.

   4. Diagnosis and Discussion.

      A case of absolute sterility from arrested development of

the genitalia. Multiple cystic degeneration of the follicles, as

described here, are frequently seen in the ovaries of young girls,

in the human family. These correspond histologically to the cystic

changes described for ovaries in other cases herein reported.

## Case 14.

   Clinical History. Holstein heifer, age 2 years. Showed normal

periods of estrum. Examination early in December 1920, to determine

inability to conceive resulted in finding an occluded vagina. This

was thought to be a case of arrested development of the genitalia.

The animal was closely bred, but not a twin. Condemned for sterility

and slaughtered for food purposes December 18, 1920.

## Necropsy

   1. Gross Description. The vagina is 7 cm., in length, the

anterior extremity being completely closed. There is no cervix uteri.

The vagina communicates anteriorly with two tubes as in case 13.

The tubes are contained within a common tough and fibrous sheath.

The tubes themselves are very fibrous. The lumen is collapsed, and

almost adherent from side to side. It does not communicate with the

cavity of the uterus or vagina. The tubes continue forward as those

described in case 13, each one ending at a partially developed uterine

horn. The right uterine horn is 8 cm., in length and 4 cm., in cross

diameter. The walls are very thin. The horn is distended to about half its capacity with a turbid, yellow, watery fluid. The left horn is extremely rudimentary. It measures 2.5 cm., in length and 1.5 cm., in cross diameter. The walls are thick and a yellow fluid is contained in the lumen. The right uterine tube is normal. The left uterine tube is short but appears normal. The right ovary is 5 cm., in length and 3 cm., in width. It contains a corpus luteum 24 mm., in width and 19 mm., in depth. Over the surface there are many small cystic follicles. The left ovary is 3 cm., in length and 2 cm., in width. It contains many small cystic follicles.

2. Microscopical Description. The persistent Müllerian tubes show a small lumen, lined with stratified ectodermal epithelium supported by a dense connective tissue of collagenous type. Occasional glands are present in this area. Smooth muscle is distributed through the lower portion, but is not great in amount. The serosa is composed of a thick layer of areolar tissue. The partially developed cornua uteri are similar in structure to those described for case 13. The uterine tubes are normal. The ovaries show many corpora albicantia distributed throughout the cortex. Primitive follicles are normal. Nearly all medium sized and large Graafian follicles show cystic degeneration, marked by a dense coagulum replacing the granular coagulum of the liquor folliculi. Ova are not seen in any of these follicles. The cells of the stratum granulosum do not appear to be damaged.

3. Bacteriological Findings.

    (1) Cultures of the cornua uteri, uterine tubes and ovaries remained sterile.

4. Diagnosis and Discussion.

Partial arrested development of genital organs and cystic degenerative changes in Graafian follicles.

## Case 15.

1. <u>Clinical</u> <u>History</u>. Aged Holstein cow, Violet. A normal calf was delivered on November 20, 1920. The parturition was difficult owing to a breach presentation. Purulent metritis and cervicitis developed following parturition. On December 6, 1920, swabs were taken from the uterus and cultures prepared. From this material the following organisms were isolated: 1. Bacillus pyogenes (Lucet), Staphylococcus aureus (Rosenbach), and Streptococcus pyogenes, (Beta type). On the same date a retained corpus luteum was palpated in the right ovary and enucleated. The right uterine horn was elongated and much enlarged, and considerable quantity of thick yellow pus could be massaged from it. The cervix was also much inflamed. The uterus was flushed with a mild antiseptic and the cervix painted with pure Lugol's solution, at ten day intervals. Improvement was continuous and the animal was pronounced ready to breed on March 31, 1921. The periods of estrum were at this time regular. Animal apparently normal, but not pregnant June 14, 1921.

2. <u>Diagnosis</u> and <u>Discussion</u>.

(1) Purulent cervicitis and metritis, acute in type, and caused by <u>B.</u> <u>pyogenes</u>, and <u>Streptococcus</u> <u>pyogenes</u>. These organisms were found on three examinations. It will be interesting to note whether changes have been produced that will render the animal permanently incapable of reproduction. It is too early at this time to make a definite prognosis, except that clinically everything seems normal.

## Case 16.

Clinical History.  Aged Guernsey cow.  Delivered a normal calf in June 1920.  The placenta was retained following which she developed a severe purulent metritis.  It received little attention until January 13, 1921.  Examination on this date revealed a deeply seated corpus luteum in the right ovary.  The cervix uteri was inflamed and the cervical canal was partially dilated.  The uterus was enlarged and flacid, and abdominal in position.  The persistent corpus luteum was enucleated on January 17, 1921.  Following this operation the uterus began to contract, and within 24 hours 4 to 5 liters of thick, creamy pus was evacuated.  The pus was very malodorous. Cultures were prepared from this discharging material.  From this exudate the following organisms were isolated: (1) Bacillus pyogenes (Lucet) and(2) Streptococcus viridans, (Alpha type).  Following the operation upon the corpus luteum the animal was very uneasy and failed to consume her evening meal.  The discharging pus continued for 3 or 4 days.  Examination on January 24th showed the uterus to be pelvic in location, almost normal excepting for a slightly en-larged right horn.  The cervix was inflamed and was painted with Lugol's solution.  The uterus was massaged.  The animal was allowed to rest one month.  On February 28th the examination showed the right horn of the uterus still slightly enlarged.  The uterus was irrigated with a 2% solution of therapogen.  Examination on March 7th, found all organs normal and cow showing symptoms of estrum.  Dis-charged from the clinic March 9, 1921, apparently recovered. Examination in May revealed the development of a right tubo-ovarian abscess.  The animal was not pregnant.

2. <u>Diagnosis</u> and <u>Discussion</u>.

Pyometra, cervicitis, and tubo-ovarian abscess, the etiology of which is (1) <u>B</u>. <u>pyogenes</u> (Lucet) and (2) <u>Streptococcus</u> <u>viridans</u> (Alpha type). Infection in this case has probably produced changes that will lead to permanent sterility. The case should be followed, but time will not permit the report on final results in this paper.

## Case 17.

1. <u>Clinical History.</u> Holstein cow, about 5 years of age. Normal calf was born November 15, 1920. Following parturition the placenta was retained. Purulent metritis developed but received no special attention until March 28, 1921. Examination revealed a much enlarged uterus (pyometra), cervicitis, cystic left ovary and corpus luteum in right ovary. The cyst was ruptured and the corpus luteum enucleated on this date. Pus began to flow freely from the vagina within 24 hours after enucleating the persistent corpus luteum. The removal of the corpus luteum allowed the uterus to undergo normal involution. ᵀhe pus which came from the uterus was yellow in color and streaked with blood and was very malodorous. Swabs were taken from the uterus and cultures prepared. The following organisms were isolated (1) Bacillus pyogenes (Lucet), and (2) Staphylococcus epidermidis (Gordon). The uterus was washed with 2% solution of therapogen on this date, and after being emptied, the entire organ could be placed beneath the hand, by rectal palpation. Examination on April 1, 1921, showed the uterus to have better muscular tone. The cervix was still slightly inflamed. The cervix was treated with Lugol's solution. Examination on April 2d, showed the animal to be in estrum. The left horn was normal, the right uterine horn was

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still slightly inflamed.   Examination on April 4th, showed the left
horn to be practically normal, and the right uterine horn still
enlarged and flacid.  On this date the uterus was massaged and the
cervix was **treated with Lugol's** solution.    Examination on April
7th showed the right uterine horn to be smaller and with better
muscular tone.  The cervix was treated with Lugol's solution and the
uterus washed with 2% solution of therapogen.  Following this it was
massaged.  On April 9th, examination revealed a normal uterus, a
corpus luteum in left ovary, a contracted cervix, and a small
quantity of pus in the vagina.  The corpus luteum was enucleated.
The animal appeared in estrum following this and was discharged from
the clinic soon after.  She has been bred but it is not known whether
conception has taken place.

   Diagnosis and Discussion.

      Pyometra and cervicitis following parturition, and caused
by B. pyogenes and Staphylococcus epidermidis.  The animal has
apparently recovered.

### Case 18

   1. Clinical History.  Holstein cow, age 6 years.  Delivered a
normal calf November 28, 1920.  Following parturition the animal
retained the placenta.  The placenta was removed promptly.  Following
this the animal did not appear in estrum.  While decumbent a thick,
creamy discharge could be seen coming from the vagina.  The animal
was presented in the University clinic for treatment on March 28, 1921.
Examination on March 28th, revealed a large, elongated uterus, hang-
ing down over the brim of the pelvis.  In the right ovary there was a
large persistent corpus luteum.  This was enucleated.  On the follow-
ing day the animal began to discharge large quantities of pus from

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the uterus. It was thick and creamy in consistency and light yellow in color. Swabs were taken from the uterus at this time and cultures prepared. The following organisms were isolated: (1) Bacillus pyogenes (Lucet) (2) Streptococcus viridans (Alpha type) (3) Streptococcus (Gamma type) (4) Staphylococcus aureus (Rosenbach), and (5) Staphylococcus epidermidis (Gordon). The uterus gradually returned to normal. On April 22d, a clinical examination of the genitalia was made. The right horn of the uterus was slightly enlarged and upon massage a small quantity of pus could be expressed. The uterus was washed with a 2% solution of therapogen. On April 25th, the uterus was found practically normal. The horns were of equal size. The organ was massaged per rectum until a clear mucus was discharged from the vagina. The cervix was still slightly inflamed and the cervical canal was partially open. The patient was pronounced practically normal and discharged from the clinic on the following day. The animal has been bred, but it is not known whether pregnancy has resulted.

2. Diagnosis and Discussion.

Pyometra and cervicitis caused by B. pyogenes, Streptococcus viridans and staphylococci. Recovery has apparently been complete, but definite knowledge of this cannot be had until later, when it is definitely known that a fetus has been carried through the normal period of gestation.

## Case 19.

Clinical History. Cow without clinical history. Specimen was obtained from the abattoir at South St. Paul.

### Necropsy

1. Gross Description. The vagina is normal. The cervix is

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normal. The right horn of the <u>uterus</u> is approximately 8 cm., in diameter, at a point just anterior to the corpus uteri. It is hard upon palpation. It gradually tapers toward the tip of the horn. The lumen of the horn contains about 100 cc., of a yellowish, granular exudate. The mucosa (endometrium) and myometrium are extensively invaded with small yellow tubercles. Many are caseous and others are calcified. On the mucous membrane these form small yellow, hard, rounded prominences. The <u>left uterine horn</u> is normal in size, except near the tip where there is a hard mass 4 cm., in diameter, and 2 cm., in length. This contains many small calcified tubercles. There is no exudate in the lumen of this horn. The <u>right tuba uterina</u> is normal in appearance at the isthmus. Toward the ampulla it is dilated to 4 mm., and is very hard upon palpation. It is yellow in color. The <u>left tuba uterina</u> presents a similar involvement. The <u>right ovary</u> contains a cyst 17 mm., in diameter, and at the opposite end of the ovary, a large corpus luteum. The corpus luteum is 2 cm., in diameter. The center presents a cystic cavity 4 mm., in diameter. The <u>left ovary</u> is normal. There are many soft tubercles, gray in color, upon the brood ligament and surrounding the uterine tubes and ovaries. The mass is so dense that dissection of these organs from it is rendered very difficult.

2. <u>Microscopical Description</u>.

The <u>uterus</u> shows many tubercles throughout the wall, especially in the endometrium. The smaller ones have no central necrosis. These are composed of endothelioid cells and many giant cells are also seen. A zone of lymphocytes surrounds the tubercle. Larger ones are similar, with central necrosis. The stroma of the endometrium is infiltrated densely with lymphocytes. The glands are

for the most part normal. A few are dilated, showing a thinned epithelium and fibrous thickening of the membrana propria. In the muscularis several large necrotic tuberculous masses are seen. The central portion shows extensive calcification, surrounding which is a wide area of necrosis. Around the necrotic area there are massed large numbers of endothelioid cells and many giant cells, intermixed with and especially massed at the outer periphery there are numerous lymphocytes. The larger tubercles are well encapsulated with connective tissue. The larger tubercles of the muscularis extend also into the endometrium almost to the uterine epithelium.

Areas of the uterus where no tubercles appeared to be present grossly, are also normal microscopically.

The uterine tubes are practically destroyed by the tuberculous process. The tubes are slightly larger than normal. The mucosa is entirely destroyed. No normal structures remain.     There is necrosis of all layers to the muscular coat. Around the necrotic material a thick layer of endothelioid cells is prominent, through-out which a few lymphocytes and fibroblasts are seen. A few giant cells are also  present. The outer circular muscular fibers are still intact.    Some sections show portions of tubal epithelium in a fairly good state of preservation, and others with necrotic remnants still present. In these areas there is extensive lympho-cytic infiltration of the mucosa, with exudate consisting of coagulated serum and lymphocytes in the lumen. The serosa shows numerous tuberculous adhesions about it.

3. Diagnosis. Advanced miliary tuberculosis of the uterine tubes, uterus and parauterine tissues.

### Experimental Inoculation with Bacillus Pyogenes.

Two heifers were secured for experimental work with Bacillus pyogenes. An attempt was made to infect these animals with recently isolated strains of the organism. Heifer No. 1 was in the eight month pregnancy, when 5 cc., of a serum bouillon culture of Bacillus pyogenes from case 17 was introduced into the jugular vein (3 weeks before parturition). No observable symptoms of disease resulted. The animal carried the calf to maturity and two weeks following parturition the necropsy revealed a normal genital tract, free of bacteria. No other pathologic lesions were found. Heifer No. 2 was non-pregnant and 15 cc., of a bouillon culture of Bacillus pyogenes from case 18 was introduced into the uterus. The animal developed no observable symptoms of disease and necropsy revealed a normal genital tract, practically free of bacteria. These cases illustrate the resistance of the normal body to infection, and the necessity of a predisposing cause. The prevalence (of B. pyogenes) in nature is not known. It must inhabit the animal's body and develop pathogenic characters when resistance for some reason or other is reduced. The inability to interfere with pregnancy in the pregnant animal is also noteworthy.

Laboratory animals were not inoculated with Bacillus pyogenes. Time did not permit pathogenicity studies on small animals. The pathogenicity of B. pyogenes for laboratory animals has been thoroughly demonstrated. According to Berger[63] and Holth,[64] rabbits are the most susceptible of the small laboratory animals to experimental infection with Bacillus pyogenes. Guinea pigs are less susceptible, and mice least so. Brown and Orcutt were able to infect rabbits quite readily, the characteristic lesions produced being abscesses in various parts

of the body.

## Summary and Conclusions.

### 1. Bacteriological.

In the course of the bacteriologic investigations, standard works have been consulted for classification. Each organism was isolated in pure culture and then subjected to systematic study for identification. In the study of staphylococci the work of C. E. A. Winslow, et al[56], on "Notes on the Classification of the White and Orange Staphylococci", has been the basis of identification. Streptococci were at first identified according to Hollman's[57] classification and in the later work the identification was based solely on the reaction to blood agar. For the latter the monograph of James Howard Brown[58] has been followed carefully. For the identification of Bacillus pyogenes the work of Brown and Orcutt on "A study of Bacillus Pyogenes" has been the basis of classification.[48] In addition to this, two rabbits were immunized with separate strains of Bacillus pyogenes. Serum from these rabbits agglutinated all strains of the organism to an equal degree. The following tables show the prevalence of organisms as they appeared in the 19 cases studied:

## Table 1.
### Compilation of Bacteriologic Results

| Genital organ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Case Number | | | | | | | | | | | | | |
| Vagina | 1 5 14 | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Os uteri externum | 1 5 14 | X | X | X | X | 1 2 5 | 1 10 13 | 1 2 4 | 3 | 0 | X | X | Y | Y | 11 13 | 13 15 | 13 15 | 1 3 13 15 19 | X |
| Corpus uteri | 0 | 3 5 12 13 | 0 | 0 | 0 | 1 2 5 | X | 1 2 4 | 0 | 0 | 1 3 15 17 19 | 0 | Y | Y | 11 13 | 13 15 | 13 15 | 1 3 13 15 19 | 20* |
| Right cornu uteri | 0 | 3 5 12 13 | 13 | 0 | 13 | 0 | 3 10 13 | 1 2 4 | 12 | 3 | " | 0 | 0 | 0 | X | X | X | X | 20* |
| Left cornu uteri | 0 | 3 5 12 13 | 0 | 0 | 0 | 0 | 3 10 13 | 1 10 | 0 | 0 | " | 0 | 0 | 0 | X | X | X | X | 20* |
| Right uterine tube | 0 | 3 5 12 13 | 0 | 0 | 4 9 13 | 0 | 3 10 13 | 0 | 0 | 0 | 0 | 0 | X | 0 | X | X | X | X | 20* |
| Left uterine tube | 0 | 3 5 12 13 | X | X | 0 | 0 | 3 10 13 | 0 | 0 | 0 | 0 | 0 | X | 0 | X | X | X | X | 20* |
| Right ovary | 0 | 2 12 | 0 | 0 | 0 | 0 | 3 10 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | X | X | X | X | X |
| L. ovary | X | 0 | X | X | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | X | X | X | X | X |
| Ovarian cysts | 0 | 0 | 0 | 0 | 13 R. | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | X | X | X | X | X |
| Ovarian abscess | Y | 2 12 | Y | Y | 13 9 | Y | 3 10 13 | Y | Y | Y | Y | Y | Y | Y | X | X | X | X | X |
| Peri-uterine abscess | Y | Y | 7 13 | 4 8 13 16 | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | X | X | X | X | X |

### Explanation of Symbols

| | |
|---|---|
| 0 - sterile | 10 - Strep. non-hemolyticus ii |
| X - not cultured | 11 - Strep. pyogenes |
| Y - structure not present | 12 - Strep. mitis |
| 1 - Staphylococcus aureus | 13 - B. pyogenes |
| 2 - Staphylococcus aurantiacus | 14 - B. subtilis |
| 3 - Staphylococcus epidermidis | 15 - Strep. viridans |
| 4 - Staphylococcus candidus | 16 - B. proteus |
| 5 - B. coli communis | 17 - Sarcina lutea |
| 6 - B. coli communior | 18 - Torula rosea |
| 7 - Strep. anginosus | 19 - Strep. (Gamma type) |
| 8 - Strep. fecalis | 20 - B. tuberculosis |
| 9 - Strep. ignavus | * - stain only |

## Table 2
### Summary of Bacteriologic Results including all cases.

| Organism | Times found | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strep. Alpha type | 8 | - | + | - | + | + | - | - | - | + | - | + | - | - | - | - | + | + | + | - |
| Strep. Beta type | 2 | - | - | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - |
| Strep. Gamma type | 4 | - | - | - | - | - | - | + | + | - | - | + | - | - | - | - | - | - | + | - |
| B. pyogenes | 9 | - | + | + | + | + | - | + | - | - | - | - | - | - | - | + | + | + | + | - |
| Staph. aureus | 6 | + | - | - | - | - | + | + | + | - | - | + | - | - | - | - | - | - | + | - |
| Staph. all other types | 10 | - | + | - | + | + | + | + | + | + | + | + | - | - | - | - | - | - | + | - |
| B. coli | 3 | + | + | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| B. tuberculosis | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | +* |
| B. proteus | 1 | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Sarcina lutea | 1 | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - |
| Torula rosea | 1 | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - |

*B. tuberculosis, by stain; organs not cultured.

Table 3.
Summary of Bacteriologic Results in Cases of Suppuration

| Organism | Times found | Case number | | | | | | | | | | | Percent present |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2 | 3 | 4 | 5 | 7 | 11 | 15 | 16 | 17 | 18 | 19 | |
| B. pyo-genes | 9 | + | + | + | + | + | - | + | + | + | + | - | 81.8 |
| Strep. Alpha type | 7 | + | - | + | + | - | + | - | + | + | + | - | 63.6 |
| Strep. Beta type | 2 | - | + | - | - | - | - | + | - | - | - | - | 18.1 |
| Strep. Gamma type | 3 | - | - | - | - | + | + | - | - | - | + | - | 27.2 |
| Staph. aureus | 3 | - | - | - | - | + | + | - | - | - | + | - | 27.2 |
| Staph. other types | 6 | + | - | + | + | + | + | - | - | - | + | - | 54.5 |
| B. coli | 2 | + | - | - | - | + | - | - | - | - | - | - | 18.1 |
| B. tuber-culosis* | 1 | - | - | - | - | - | - | - | - | - | - | + | 9.0 |
| B. proteus | 1 | - | - | + | - | - | - | - | - | - | - | - | 9.0 |

* By stain only; cultures not made.

Case 11, mild puerperal metritis of short duration; healing would probably soon have occurred.

Case 18, tuberculous metritis and salpingitis.

It is confusing in interpreting results in table 1 to have such a large group of Streptococci. All strains, excepting <u>Streptococcus pyogenes</u> and <u>Streptococcus anginosus</u>, are of the non-hemolytic (Alpha and Gamma types). These two, however, fall into the group of hemolytic streptococci, (Beta type).

It will be seen in careful study of the material presented that the organisms most important in infections of the genital tract of the cow are <u>Streptococci</u> and <u>B</u>. <u>pyogenes</u>. These types are responsible for the greater number of infections. In all cases of severe non-specific metritis and other suppurative processes about the genitalia <u>Bacillus</u> <u>pyogenes</u> combined with streptococci, or the latter alone, have been isolated. Other organisms are not regarded as playing a primary role. Unless the bacteriologic study is made during the progress of active infection, these types as well as others will most often not be found. In such cases one views only the results of infection, the active process having subsided. It is for this reason, that in nearly all cases when no gross evidence of active inflammation exists, the genital tract is sterile. The vagina, cervix uteri and corpus uteri must be excepted.

Organisms are not harbored as commensals normally in the internal genitalia of the cow, with exceptions as already made. Case 6 is an unparalleled example of this. Hydrosalpinx, the end result of active inflammation existed, cultures from which were sterile. Case 10 also illustrates the point. Many other examples of a bacterial free genital tract may be found in the case reports, and as compiled in table 1. The absence of <u>B</u>. <u>abortus</u> (Bang) supports the previous view, that it is not found in puerperal infections that persist for any length of time, and that it does not persist, so far as is known.

in the genitalia of the cow. B. abortus was not isolated from any
of the cases studied.

2. Pathological.

The frequency with which one views degenerative changes
in the ovaries of sterile cows cannot be disregarded. It seems that
in all cases of infection about the genital tract, multiple cystic
degeneration of medium sized Graafian follicles is an almost constant
finding. Until more study is made, especially on normal animals as
controls, no definite statement can be made. The uterine tubes,
because of their many folds and crypts and their delicate structure,
offer the most favorable site for persistence of infection. Chronic
changes in the genital tract are most frequent in this location and
are most always seen in the form of hydrosalpinx. In the uterus
chronic changes do not readily occur. Only after severe infection
over a long period of time are sufficient changes produced to render
the organ incapable of serving its natural use. It does not seem as
readily susceptible to persistence of infection as do the uterine
tubes. The changes noted in the uterus are fibrosis, dilation of
glands, atrophy of glandular epithelium, thickening of uterine
epithelium, etc. The cervix uteri like the uterine tubes offers a
favorable site for bacterial reproduction. The folds of the mucosa
are deep and in the crypts bacteria may gain entrance and multiply.
Although accessible to treatment the deep crypts allow infection to
persist and often extend to other portions of the genital tract.
Hypertrophy of cervical folds is the most constant chronic change.
The vagina because of its smooth stratified epithelial surface is not
readily infected. It may harbor pathogenic types of bacteria, as
commensals, on its surface, in its accessory glands, in the sub-

epithelial lymph follicles, or in the urethral orifice.    It is
suggested that the vagina and accessory structures are the reservoir
for organisms which infect the genital tract of the cow, including
B. abortus, and other types which cause congenital (prenatal)
infections.    The route of infection may be through the lymphatic
system, which is most highly developed about the female genital
tract.    The occurrence of para and peri uterine infections seems to
support this view.

A total of nineteen cases has been studied and presented.    The
theory of infection does not explain all cases of sterility.    Where
no evidence of infection, past or present exists, and the animal
is sterile the ovaries must be looked to as the cause of the
condition.
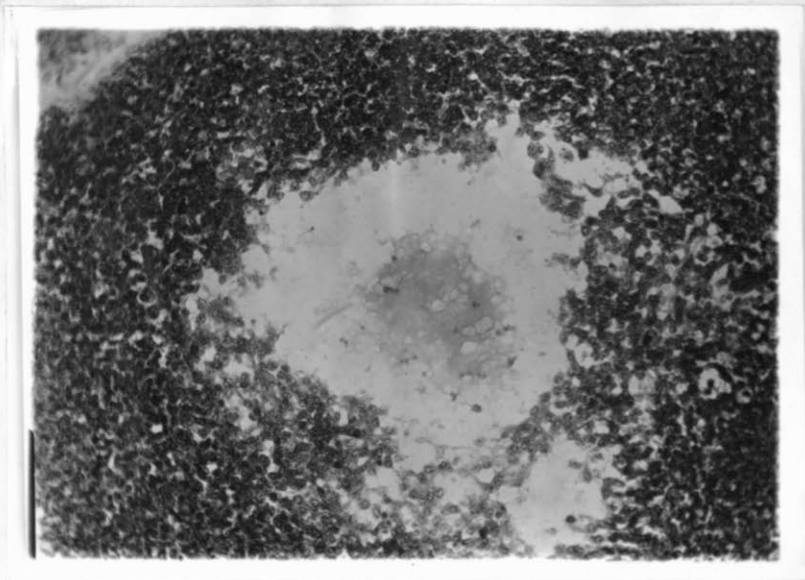
Fig. 1. Degenerative change in Graafian follicle, Case 5. Note proliferation of cells of stratum granulosum.
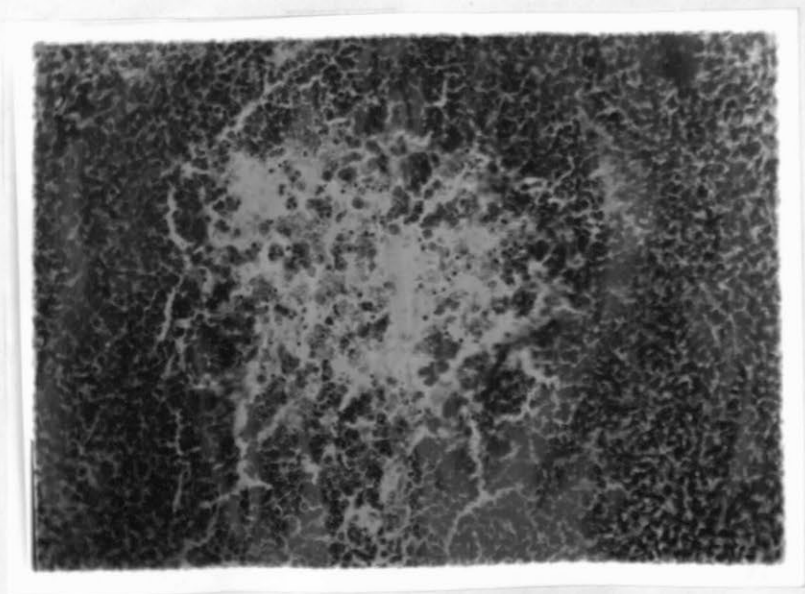


Fig. 2. Graafian follicle, Case 1, showing degenerative change. Note thickening of stratum granulosum and theca folliculi.

Fig. 3. An example of cystic degeneration
of Graafian follicle, Case 5. Note dense
coagulum replacing normal liquor folliculi
and degenerative changes in cells of stratum
granulosum (not marked in this case).
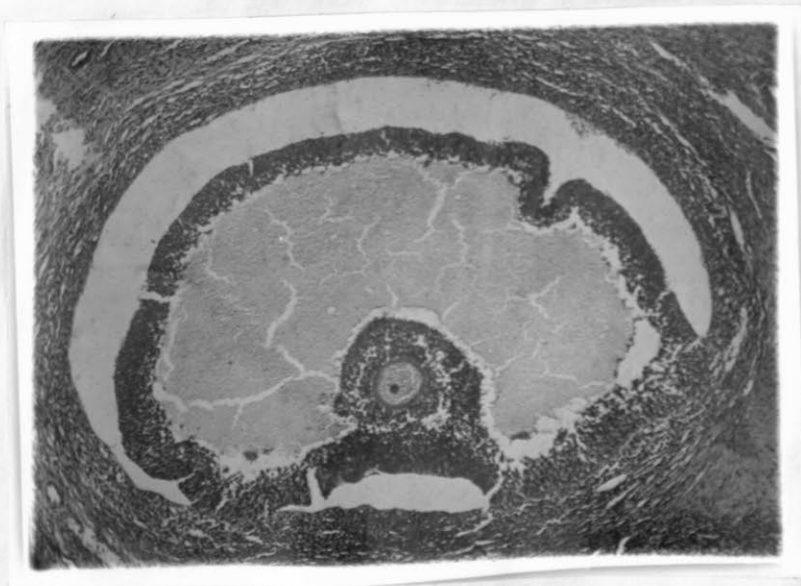


Fig. 4. Example of normal Graafian folli-
cle. Note the granular character of the
coagulated liquor folliculi. The pulling of
the stratum granulosum from the theca folliculi
is an artefact. Case 3.

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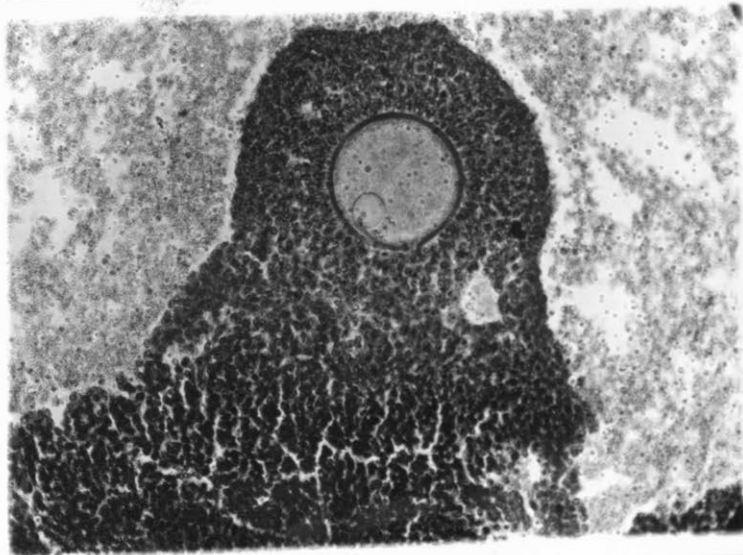
Fig. 5. Normal follicle, Case 2., show-
ing ovum surrounded by the cells of the
cumulus oöphorus. Note the granular char-
acter of the liquor folliculi coagulum.
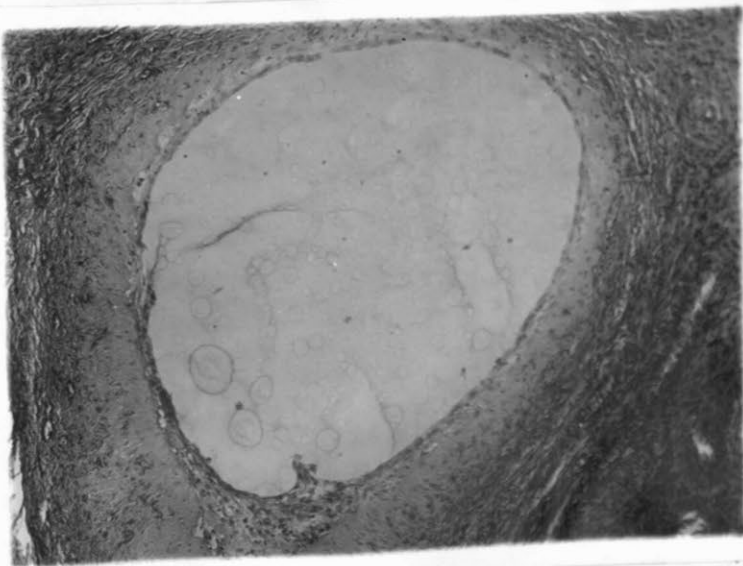


Fig. 6. A small cystic corpus luteum.
Lutein cells have undergone hyaline change.
Cyst surrounded by corpus albicans. Fluid
contains neutral fat. Case 5.
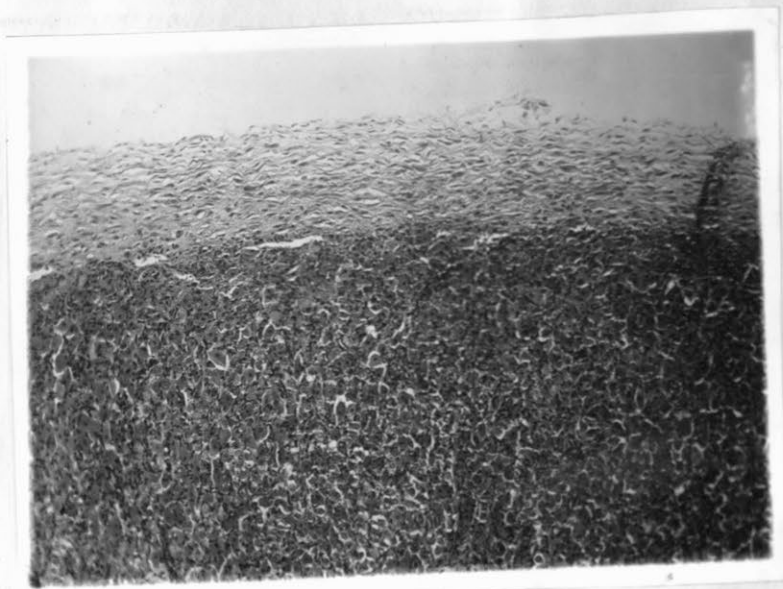
Fig. 7.  Section through wall of old
corpus luteum cyst.  Note connective tissue
and lutein cells.  Case 10.
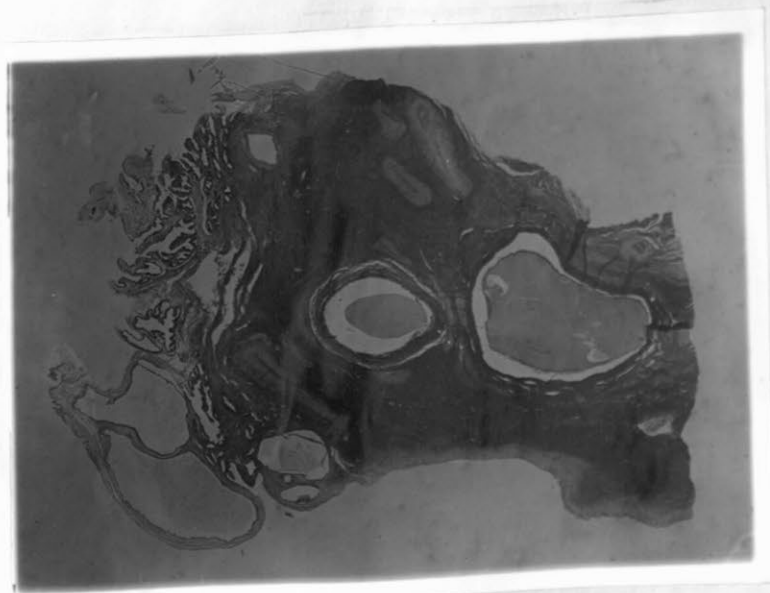


Fig. 8.  Section through ovary, Case 9.
Note uterine tube adherent to ovary, the
multilocular cavities characterizing hydro-
salpinx.  Two Graafian follicles showing
cystic degeneration are seen in the ovary.

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Fig. 9.    Low power of uterine tube,
Case 5.    Hydrosalpinx, early stage.



Fig. 10.    Hydrosalpinx, Case 5.

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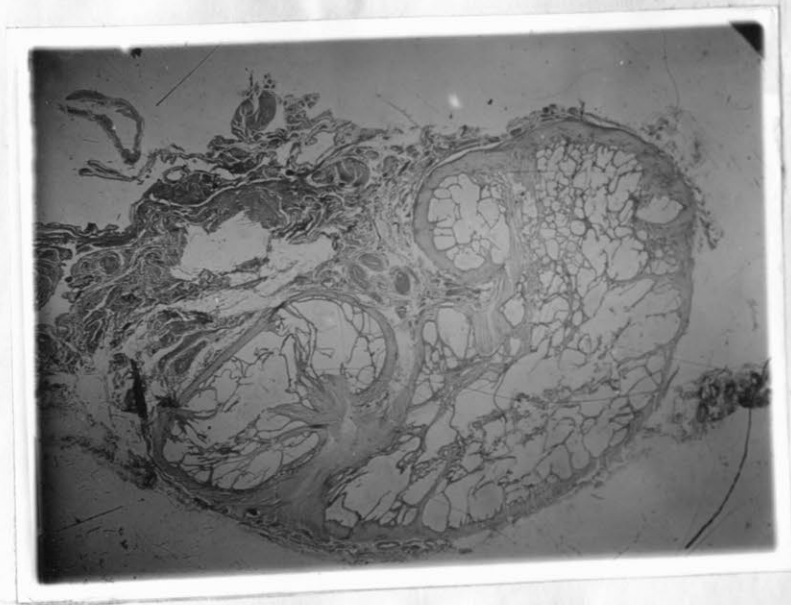
Fig. 11.   Section through uterine tube,
Case 6.  Hydrosalpinx, later stage.  Note
atrophy of many interlocking folds.
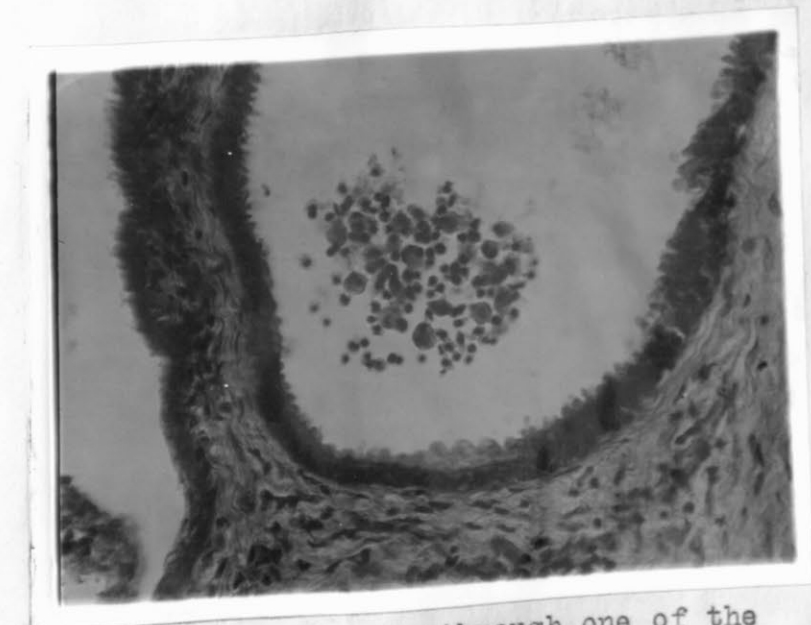


Fig. 12.   Section through one of the
cystic pockets.  Hydrosalpinx, Case 5.
Note proliferation of connective tissue
and epithelium.  Exudate in lumen is com-
posed of polymorphonuclears, lymphocytes
and desquamated epithelium.

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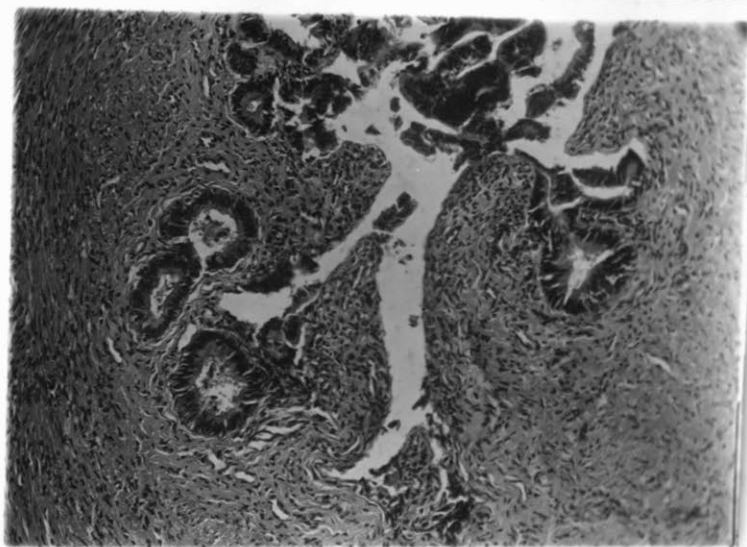
Fig. 13. Mild salpingitis, Case 4.
Epithelium is abnormally desquamated and
in the wall many lymphocytes are seen.
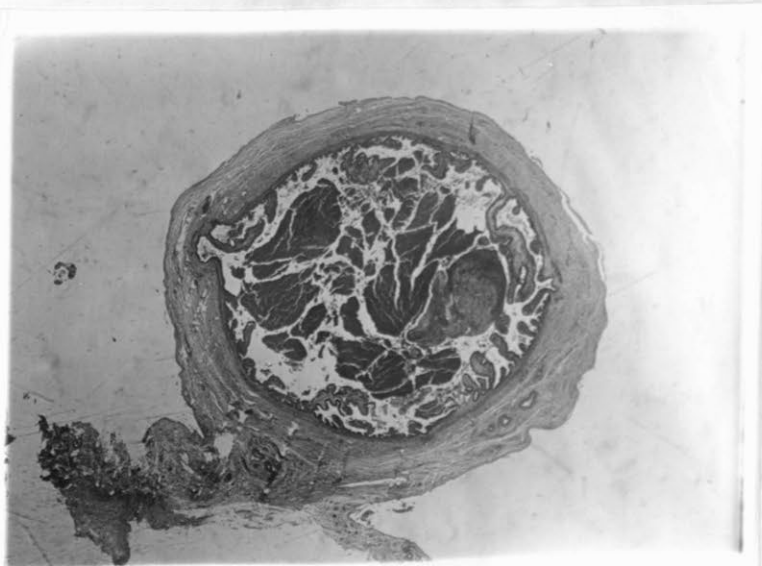


Fig. 14. Purulent salpingitis, Case 2.
Low power section of uterine tube. Note
the purulent exudate in the lumen.
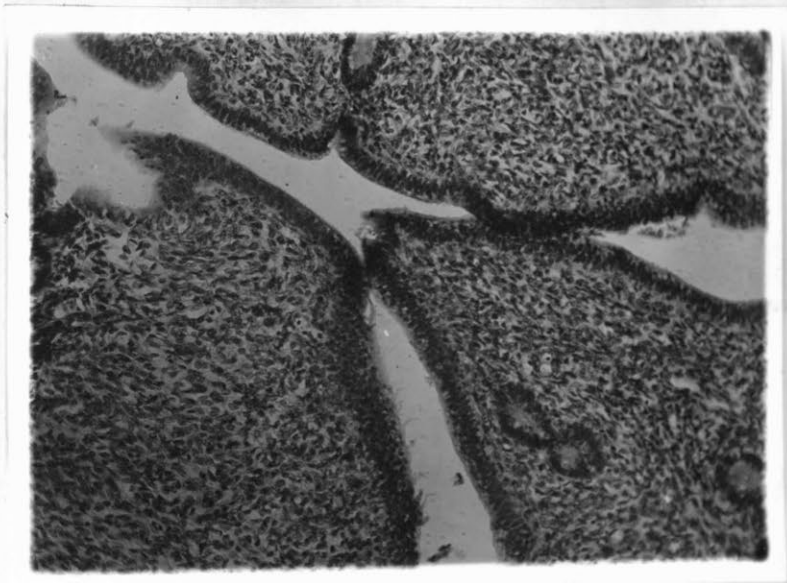
Fig. 15. Section through wall of cornu uteri, showing some thickening of uterine epithelium. Case 6.



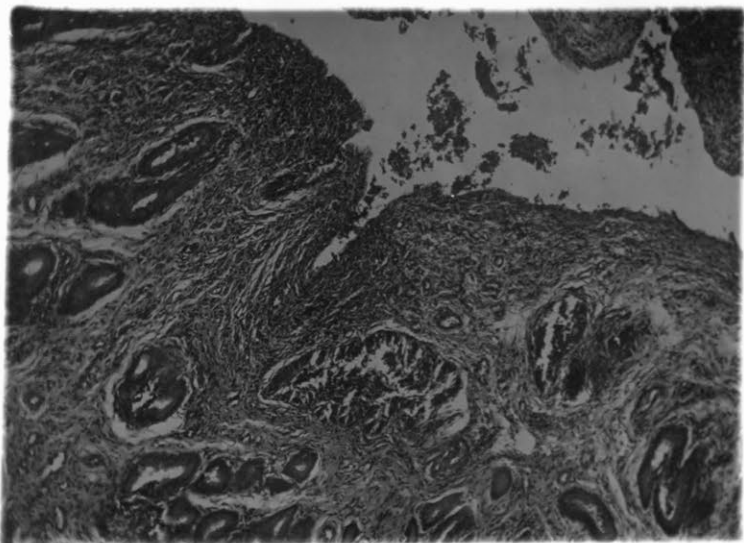Fig. 16. Section through caruncula, Case 7. Note great thickening of uterine epithelium. Stratified over caruncula and simple columnar at the border. "Ectodermoisering."

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Fig. 17. Section through wall of cornu
uteri, showing necrosis of many superficial
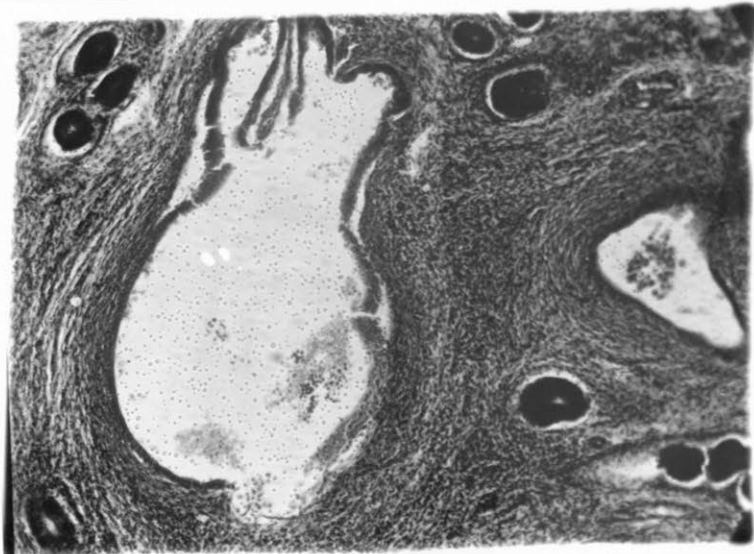uterine glands. Case 3.



Fig. 18. Dilated uterine glands, Case 2.
There is considerable destruction of the
glandular epithelium. Other deeply stained
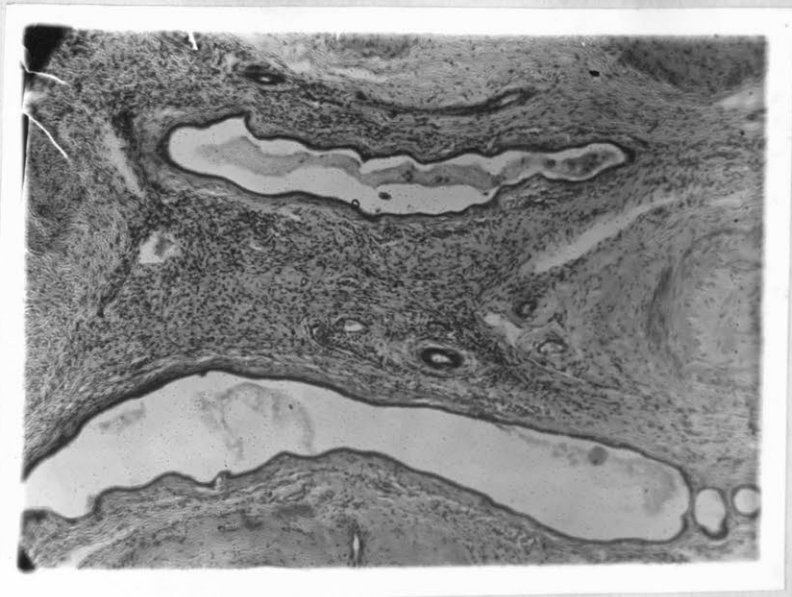glands in same section remain normal.

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Fig. 19. Section through wall of uterus,
Case 7. Note fibrosis of uterine stroma,
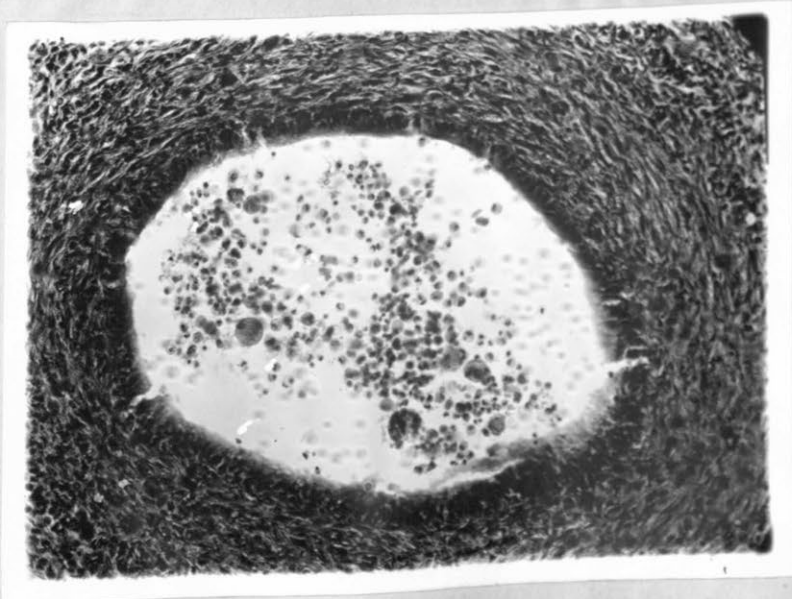dilation of uterine glands and atrophy of
glandular epithelium.



Fig. 20. Section through dilated uterine
gland, Case 2. Note connective tissue pro-
liferation surrounding gland and exudate
in its lumen, mostly polymorphonuclears.

Fig. 21.  Normal fold in cervix uteri,
Case 5.  These folds may harbor pathogenic
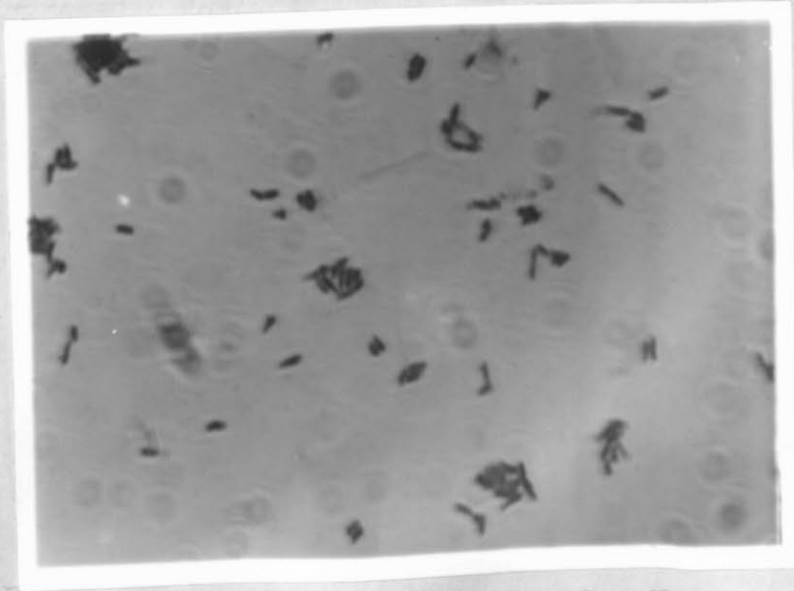bacteria; in them infection is persistent
when once started.



Fig. 22.  B. pyogenes, Case 18.  Note
the characteristic clusters, parallel grouping
and diphtheroid types.

Fig. 23.   Gross specimen, genitalia,
Case 4.   Shows extensive periuterine abscesses.

Fig. 24. Maldeveloped genitalia, Case 13.

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In the preparation of the embryology, gross and histological anatomy of the genital tract of the cow, liberal use of standard texts has been made, and for this I wish to give due credit. The following works have, in many cases, furnished material almost without change:

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Bovine Obstetrics, M. G. DeBruin.

Die Sterilitat des Rindes, E. Hess.

Epizootic Abortion in Cows, S. Wall.

Handbuch der vergl. Mikroskop. Anat., W. Ellenberger.

Text-Book of Embryology, Bailey and Miller.

Text-Book of Embryology, C. W. Prentiss.

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# Bibliography

1. Dorland, W. A. N., American Illustrated Medical Dictionary, W. B. Saunders & Co., Phila., 1915, 926.

2. Bang, B., Die Aetiology des seuchenhaften (infectiosen) Verwerfens. Zeitschr. f. Tiermed, 1897, 1, 241-278.

3. A Bibliography - Contagious Abortion of Cattle, Vet. Rev. Lond., 1919, 3, 137.

4. Complete Farmer, 5th Edition, 1807.

5. Nocard, Recherches sur l'avortment epizootique des vaches, Rec. Méd. Vet. 1886, 3, 669.

6. Woodhead, G. S., et al, First Report, Committee of the Highland and Agricultural Society of Scotland, Jour. Comp. Path. and Therap., 1889, 11, 97.

7. Board of Agriculture and Fisheries, Report of the Departmental Committee Appointed to Inquire into Epizootic Abortion, Part I and Appendix, 1909.

8. MacNeal, W. J., and Kerr, J. E., Bacillus Abortus Bang the Cause of Contagious Abortion in Cattle, Jour. Inf. Dis., 1910, 7, 469.

9. Eberhard and Gunther, Verhandeling over het Verlassen der Koeyen, Amsterdam, 1793.

10. Skellet, A Practical Treatise on the Parturition of the Cow, etc. Lond. 1907.

11. Fleming, G., A Text-Book of Veterinary Obstetrics, American Edition, Wm. R. Jenkins, N. Y. 1889.

12. Hill, W. J., The Principles and Practice of Bovine Medicine and Surgery, Baillière, Tindall and Cox, Lond., 1882.

13. Dieckerhoff, W., Lehrbuch der Speciellen Pathologie und Therapie f. Thierärzte, Erster Band, 2nd. Ed. 1892, 966, 967.

14. Cook, J., Endometritis in Heifers, Jour. Comp. Path. and Therap., 1891, 4, 68.

15. Awde, W., Endometritis in Heifers, Jour. Comp. Path. and Therap., 1891, 4, 164.

16. Wall, S., The Alterations in the Uterus in Epizootic Abortion and in other Infectious Metrites in Cows, Tenth Internat. Vet. Congress, Lond., 1914, 292.

17. Sobotta, Histologie und mikroskopische Anatomie des Meuschen, 2te
        Auflaug, 1911, 197, Note 4 (Quoted from S. Wall).

18. Bailey, F. R., Miller, A. M., Text-Book of Embryology,
        Wm. Wood & Co., N. Y., 1911, 413.

19. Albrechtsen, J., Sterility of Cattle and Methods of Treatment,
        The Cornell Vet., 1917, 7, 95.

20. Hess, E., Die Sterilitat des Rindes ihre Erkennung und Behandlung,
        M. & H. Schaper, Hannover, 1920.

21. Fleming, G., Flemings Veterinary Obstetrics, 3rd Ed., Revised by
        J. F. Craig, Wm. R. Jenkins, N. Y., 1912, 51.

22. DeBruin, M. G., Bovine Obstetrics, Wm. R. Jenkins, N. Y., 1901,
        2, 16, 18.

23. Heitz, Fr., Über den Bau der Kalbsovarien, Berner Inaugural
        Dissertation, 1906, und Archiv. f. Wissensch. und
        prakt. Tierhkde., 1906, 32.

24. Kapelli, J., Beiträge zur Anatomie und Physiologie der Ovarien
        von wildledenden und gezähmten wiederkäuern und
        Schweinen. Berner Inaugural Dissertation, 1908.

25. Sisson, S., Veterinary Anatomy, W. B. Saunders Co., Phila.,
        1911, 517, 519.

26. Williams, W. L., Veterinary Obstetrics, Ithaca, N. Y., 1909, 28.

27. Wall, S., Epizootic Abortion in Cows, etc., 294.

28. ibid, 296.

29. Ellenberger, W., Handbuch der Vergleichenden Mikroskopischen
        Anatomie der Haustiere, Berlin, 1911, 588, 488, 572.

30. Hess, E., Die Sterilitat des Rindes, Hanover, 1920, 7.

31. Palmer, F. E. and Eckles, C. A., Jour. Biol. Chem., 1914, 17, 194.

32. Heape, Phil. Trans. B., 1897, CLXXXVIII, 135.

33. Marshall, F.H.A., The Physiology of Reproduction, Longmans & Co.,
        1910.

34. Marshall, F. H. A., and Runciman, J. G., On the Ovarian Factor
        Concerned in the Recurrence of the Estrous Cycle,
        Jour. Physiol., 1914, 49, 12.

35. Fraenkel, Arch. F. Gynak., 1903, LXVIII, 438.

36. Boyd, W. L., University of Minnesota, (Personal communication).

37. Pearl and Surface, Effect of Corpus Luteum on Ovulation,
        Journal of Biological Chemistry, 1914, 19, -263

38. Gebauer, Sterility in Cows, Abst. from Deutsche tier Wochenschr.
        in Am. Vet. Rev., 1914, 45, 268.

39. Williams, W. L., Veterinary Obstetrics, 1909. (Quoted from p. 161).

40. Kitt, Th., Pathologische Anatomie der Haustiere, 4th Auf.
        Bd. II, S. 637.

41. Rasenau, E. C., and Davis, C. H., The Bacteriology and Experiment-
        al Production of Ovaritis, Jour. Am. Med. Ass.
        1914, LXIII, 903.

42. Fitch, C.P., A Preliminary Study of the Pathology and Bacteriology
        of Ovaritis in Cattle, Report N. Y. State Vet. Coll.,
        1915-16, 199.

43. Loeb. Leo., The Experimental Production of Hypotypical Ovaries
        Through Underfeeding. A Contribution to the Analysis
        of Sterility., Biological Bulletin, 1917, 32.

44. Gillman, H. L., The Diseases of the Oviduct of the Cow and their
        Relation to Sterility, Cornell Vet., 1921, 11, 4-20.

45. Eggink, B., Inaugural dissertation, Aetiologie der Metritis beim
        Rinds, Sneek, 1914.

46. Lucet, A., Recherches bactériologiques sur la Suppuration chez les
        animaux de l'espece bovine, Ann. Inst. Pasteur, 1893,
        7, 325.

47. Kunnemann, O., Ein Beitrag zur Kenntniss der Eitererreger des
        Rindes, Arch. wiss. u. prakt. Tierheilk., 1903, 7, 325.

48. Brown, J. H., and Orcutt, M. L., A Study of Bacillus Pyogenes,
        Jour. Exp. Med., 1920, 32, 219-248.

49. Hess, E., Die Sterilat des Rindes, Hanover, 1920, 49.

50. Hofman, H., Über Zystenbildung in der Vagina des Rindes, Deut.
        Tierarz. Woch., 1920, 28, 621.

51. Myers, K. F., Communicated through Dr. C. P. Fitch, U. of
        Minnesota.

52. Williams, W. L., Granular Venereal Disease, Abortion and Sterility
        in Cows, Report N. Y. State Vet. Coll., 1909, 92.

4-21 6M

53. Grips, W., Über eine mit multipler Abzessbildung verlaufende
    Pleuritis und Peritonitis der Schweine und deren Erreger,
    z. Fleisch - u. Milchhyg., 1898, 8, 166.

54. Glage, F., Ueber den Bazillus pyogenes suis Grips, den
    Bazillus pyogenes bovis Künnemann und den bakteriologischen
    Befund bei den chronischen, abszedierenden Enterentz

56. Winslow, C.E.A., et. al, Notes on the Classification of the White
    and Orange Staphylococci, Jour. Bact., 1920, 5, 145.

57. Hollman, W. L., The Classification of Streptococci, Jour. Med.
    Res., 1916, 34, 377.

58. Brown, J. H., The Use of Blood Agar for the Study of Streptococci,
    Monograph Rockefeller Inst. for Med. Res. 1919, No. 9.

59. Wallace, R., The Physiology of Reproduction, by F. H. A. Marshall,
    47.

60. Ewing, J., Neoplastic Diseases, W. B. Saunders, 1919, 562.

61. Hallman, E. T., Further Studies on the Pathology of the
    Reproductive Organs in Sterility. Jour. Am. Vet. Med.
    Assoc. 1920, LVII, 8-28.

62. Williams, W. L., The Diseases of the Genital Organs of Domestic
    Animals, Ithaca, 1921, 595, 611.

63. Berger, E., Bakteriologische Untersuchungen über einige
    chronische Lungenentzündunger des Rindes, Z. Infek.
    Haustiere, 1908, 3, 356.

64. Holth, H., Untersuchungen über den Bacillus Pyogenes und die
    durch ihn hervorgerufenen gewebsveränderungen, Z.
    Infektionskrankheit., Haustiere, 1908, 3, 155.

65. Ellenberger, W., Vergleichende Physiologie der Haussaugethiere,
    Berlin, 1892, 2.

4-21 6M