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CYTOMORPHOSIS OF PORCINE SPERMATOZOA AS RELATED

TO ARTIFICIAL INSEMINATION

by

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A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of

The Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: Veterinary Anatomy

Approved:

In Charge of Major Work

Head of Major Department

Dean of Graduate College

Iowa State College

1958

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I. INTRODUCTION

In recent years, there has been a renewed interest in
artificial insemination of swine. The technique for this
procedure has been known and used experimentally for 30 years.
During this time, artificial breeding in cattle has developed
into a large enterprise. This development has not been shared
by swine artificial breeding because the storage life of boar
spermatozoa was thought to be short and the system of natural
breeding generally used in this country has been satisfactory.
With recent emphasis on the meat type hog, artificial in-
semination is being viewed as a possible aid in reaching
genetic objectives in a shorter period of time. Also, recent
trends toward integrated pork production may demand artificial
insemination of a large number of sows during a short period
of time, in order to group the pigs at farrowing time to go
along with unit production.

A knowledge of spermatogenesis, sperm-cell survival, and
fate in the female genital tract is important if swine re-
production is to be thoroughly understood. The spermatozoon
is an atypical cell. It is essentially a nucleus with a hap-
loid chromosome number functioning with very little cytoplasm.
This specialized cell is capable of independent activity but,
because of its lack of cytoplasm, it is dependent on its sur-
rounding medium for the nutritive substances necessary to
supply the energy to complete its physiological mission,

fertilization. The secretions of the female genital tract as well as those of the male genital tract undoubtedly play an important part in providing these substances. For this reason, histological and histochemical studies of both the male and female genital tract were included in this work. The accessory male genital glands were not included.

To study some of the practical aspects of artificial insemination of swine, a number of sows were inseminated with considerable effort being directed toward keeping accurate records.

This study is an attempt to determine the changes that take place during the life history of porcine spermatozoa with the hope that the findings will contribute to the development of artificial insemination in swine.

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II. REVIEW OF LITERATURE

A. Life History of Spermatozoa

1. Spermatogenesis

The process of spermatogenesis in the seminiferous tubule of the vertebrate testis has been known for many years and was well described by Metz (1932), Trautmann and Fiebiger (1949), and Maximow and Bloom (1952). The ancestors of the male germ cell are evident in the adult mammalian testis as the spermatogonia, which are the cells nearest the basement membrane of the seminiferous tubule. After a period of proliferation and growth, the spermatogonia give rise to the primary spermatocytes. The primary spermatocytes divide into two new cells, the secondary spermatocytes. Each secondary spermatocyte soon divides, giving rise to spermatids. Leblond and Clermont (1952b) classify spermatogonia into three types: type A, type B, and Intermediate. The primary spermatocytes in the rat, according to Leblond and Clermont, undergo a series of morphologically distinct nuclear forms namely; resting, leptotene, zygotene, transition form, pachytene, diplotene, diakinesis, and metaphase, before dividing into two secondary spermatocytes. Gresson and Zlotnik (1948) describe the relation of the Golgi material to spermatogenesis in the bull. Wodsedalek (1913) described these changes in the pig with special reference to the accessory chromosomes. Gresson and

Zlotnik (1945) illustrated and described spermatogenesis of the boar along with other domestic animals using hematoxylin and acid fuchsin staining.

2. Spermiogenesis

The process of differentiation of spermatids to spermatozoa is called spermiogenesis by Trautmann and Fiebiger (1949). Leblond and Clermont (1952a) describe spermiogenesis in the rat, mouse, hamster, and guinea pig, using the periodic acid-fuchsin sulfurous acid technique (hereafter referred to as PAS). These authors divide the development into 4 stages; Golgi phase, cap phase, acrosome phase, and maturation phase. Cavazos (1954) studied the histochemical reactions in vertebrate testes and described spermiogenesis in the bull and ram as studied by periodic acid Schiff technique. Leblond and Clermont (1952b) summarized and defined the stages of the cycle of seminiferous epithelium in the rat correlating the stages of spermatogenesis and spermiogenesis with the tubule cycle. Gresson and Zlotnik (1945) described the process of spermiogenesis in the boar by observations of hematoxylin and acid fuchsin preparations.

3. Epididymal spermatozoa

Mann (1954) points out changes of spermatozoa in the epididymis as a "ripening process", in which there is a migra-

tion of a drop-like swelling of sperm-cell cytoplasm called the "kinoplasmic droplet". Merton (1939) describes the kinoplasmic droplet of the mouse spermatozoa. In the head of the epididymis the droplet is located at the proximal end of the middle piece, but by the time the spermatozoa have reached the tail of the epididymis the droplets take up a position at the distal end of the middle piece and finally disappear altogether. The droplets are seldom found in ejaculated spermatozoa. Rodolfo (1934) reported a similar observation in the boar. Lasley and Bogart (1944) made a study of epididymal spermatozoa in the boar and compared it with ejaculated sperm-cells. Branton and Salisbury (1947) reported that the protoplasmic droplet migrated caudally and disappears as the spermatozoa pass through the tubular genital tract of the bull. Hancock (1957) made further studies of the cytoplasmic beads of boar spermatozoa and concluded that the cytoplasmic beads disappear when the spermatozoa are mixed with accessory fluid at the time of ejaculation.

4. **Ejaculated spermatozoa**

Studies of ejaculated spermatozoa would not be complete without some attention being devoted to the seminal plasma. White (1954) studied the effect of some seminal constituents and related substances on mammalian spermatozoa. Glover (1955) and Glover and Mann (1954) made analytical studies of

the chemical constituents of boar semen. Johari (1956) investigated the gel mass in boar semen.

There is a great fund of material on morphological studies of mammalian spermatozoa. Bowen (1924) postulated that the acrosome is essentially a secretory product, the principal function of which is to initiate physio-chemical reactions of fertilization. Green (1940) used aceto-carmine stain on sheep spermatozoa and reported a small hyaline vesicle located in the head membrane with dark field illumination. Williams (1950) gives a good morphological description for human spermatozoa. Morris (1950) reported studies of bull spermatozoa using the phase contrast microscope. Randall and Friedlander (1950) studied the microstructure of ram spermatozoa. A very good comprehensive review of the literature of the structure of mammalian spermatozoa is given by Gresson (1951). Clermont, Einberg, Leblond, and Wagner (1955) postulate that the perforatorium is an extension of the nuclear membrane of the rat sperm-cell.

Cytochemical and histochemical studies have been made of spermatozoa of many different mammalian species. Melampy, Cavazos, and Porter (1952), after histochemical studies of bull spermatozoa, reported that the postnuclear cap stained more intensely for acid phosphatase than the acrosome. The acrosome stained more intensely when stained for alkaline phosphatase. The entire head was uniformly positive for the

Feulgen reaction. The acrosome gave a weak reaction to PAS
and the cytoplasmic droplet gave a consistently strong PAS
reaction. Melampy et al. reported no glycogen in bovine sper-
matozoa by either the Bauer-Feulgen or PAS method. Wislocki
(1950) reported cytochemical reactions of human spermatozoa
and seminal plasma. Leblond, Clermont, and Cimon (1950) showed
the presence of PAS positive polysaccharides in the head cap
of rat spermatozoa, prior to their release from the Sertoli
cells. Hancock (1956) studied boar spermatozoa using stained
smears, phase contrast microscope, and the electron micro-
scope. Hancock reported that the head is approximately 8.5
micra long; twice as long as it is wide. The mid piece is
approximately 10 micra long and main tail piece is 30 micra
long. Evidence is presented to show that the acrosome has
two components; the outer acrosome is the larger, the smaller
inner acrosome is a crescentic structure. The segment-shaped
area of overlap between the two parts of the acrosome forms
the equatorial segment.

Electron microscopic studies of human spermatozoa have
been reported by Seymour and Benmosche (1941). Baylor, Nal-
bandov, and Clark (1943) reported electron microscope findings
of bovine spermatozoa. They found that the anterior portion
of the sperm-cell is always enveloped by a protoplasmic cap
which appears damaged or disappears altogether if spermatozoa
are stained or fixed. They found that the tails end in a

brush consisting of many free and very long filaments. Breaks in the main or end piece of tail have shown flared brushes which suggest that the axial filament consists of a bundle of fine fibers. Reed and Reed (1947) confirmed Baylor's et al. observations on bull spermatozoa and reported heavy granulations throughout the protoplasmic cap of some spermatozoa. They postulated that these may represent age changes in the cells. Reed and Reed (1948) compared human and bovine spermatozoa by electron microscopy. Bretschneider and Van Iterson (1947) reported electron microscopic studies of bull spermatozoa. Randall and Friedlander (1950) reported extensive work on the microstructure of ram spermatozoa, using electron micrographs. Schnall (1952) reported electron microscopic studies of human spermatozoa. Herrnleben (1955) classified goat, sheep, pig, rabbit, camel, and ass spermatozoa, according to morphology and measurements after electron microscopic studies. Kessler (1955) gives electron microscopic evidence of 11 fibrils in the sperm-cell tail. Wu and McKenzie (1955), using the electron microscope, showed the human, bull, ram, boar, and rabbit spermatozoa tails consist of nine fibrils.

A number of authors have reported results using live-dead staining techniques on smears of spermatozoa from various species. The technique is based on a background stain like nigrosin and a cytoplasmic stain like eosin. When a drop of

semen and a drop of stain are mixed and a smear prepared, the dead spermatozoa are stained and the live spermatozoa remain unstained. The following is a list of authors who have published results on this type of spermatozoa study: Lasley, Easley, and McKenzie (1944); Mayer, Squiers, and Bogart (1947); Blom (1950); Williams and Pollak (1950); Mayer and Oloufa (1951); Hancock (1951); Campbell, Hancock, and Rothchild (1953); Campbell, Dott, and Glover (1956). Mayer and Oloufa (1951) indicated that none of the live-dead staining methods they used gave consistently satisfactory results with boar semen. Apparently one of the semen plasma constituents interferes with the differential staining.

Other methods of differentiating between live and dead spermatozoa have been reported. Burgos and di Paola (1951) evaluated spermatozoa vitality by observing human spermatozoa while still motile in a dilute eosin solution. Bangham and Hancock (1955) used a filter of small glass balls. The dead spermatozoa are retained by the filter, while the live ones pass through. Bishop and Smiles (1957) differentiated living and dead spermatozoa by fluorescence microscopy.

Studies of abnormalities of spermatozoa as related to determining fertility have been recorded by several authors. Williams and Savage (1925) reported micropathological spermatozoa studies of the bull. Salisbury and Mercier (1945) published studies on the reliability of estimates of the pro-

portion of morphologically abnormal spermatozoa in bull semen and found that 100 cells, counted per slide, was sufficient sample to evaluate semen quality. Blom (1950) illustrated the medusa formation as found in bull semen as discarded fragments from ciliated epithelium of the male reproductive tract. Rollinson (1951) reported an intensive study of relationships of abnormal spermatozoa to infertility. Hancock (1953) summarized observations of spermatozoa from sterile bulls using nigrosin-eosin and Giemsa and Heidenhain's staining techniques. Frank, Benjamin, and Segerson (1954) centrifuged human semen samples, made smears, and used Papanicoloau stain to study abnormal morphology.

Semen studies directed specifically to determine the fertility of the boar have been reported by McKenzie and Phillips (1933) and Herrick (1949). Both of these authors indicate that abnormal spermatozoa approaching 25 per cent of the total count results in reduced fertility.

Morphological changes in human semen after ejaculation have been studied by Oettle (1954) using phase microscopy. Hancock (1952) reported electron microscopic studies of bull spermatozoa before and after death. He pointed out that the appearance of the acrosome after death corresponds closely to that which has previously been described as characteristic of a second head membrane, the galea capitis. It was concluded that the acrosome and galea capitis are identical.

The disintegration of bull spermatozoa was studied by Hancock (1955).

5. Spermatozoa deposited in female genital tract

Some work has been recorded on the passage of spermatozoa in the female genital tract. Mann, Polge, and Rowson (1956) studied this in the sow and the horse. The rapid movement of spermatozoa in the genital tract of the sow has been studied by Burger (1952) and du Mesnil du Buisson and Dauzier (1955a). du Mesnil du Buisson and Dauzier found that spermatozoa reached the foot of the ampulla of the oviduct in two hours and the top in 5 hours. Spermatozoa passage took longer at the beginning of estrus (8 hours) than at the end (two hours). Only a few spermatozoa reached the upper portion of the uterine horns. du Mesnil du Buisson and Dauzier (1955b) reported that within 5 hours after breeding the accessory seminal secretions were resorbed and a large percentage of the spermatozoa disappeared, presumably as a result of enzymatic digestion. No viable spermatozoa were found 50 hours after service. Lewis (1911) studied the vitality of sperm-cells within the body of the sow. He found that 80 per cent of the sperm-cells were dead after 16 hours or more had elapsed between service and slaughter.

6. Fate of spermatozoa in female genital tract

The fate of spermatozoa in the uterus of the mouse and rat has been recorded by Austin (1957). He studied the uterine fluid of mice killed two to 6 hours after coitus and ovulation, and at 14-18 hours after coitus. He found that the number of leucocytes increased and number of spermatozoa decreased as time elapsed following breeding. Phagocytosis of spermatozoa was observed. The phagocytes were nearly all polymorphonuclear leucocytes. Active phagocytosis was studied by phase microscopy and by fixed stained smears. Mann, Polge, and Rowson (1956) found that 40 minutes after mating of sows the uterine horns were filled with semen containing spermatozoa, but that 6 hours after mating, the bulk of spermatozoa and seminal plasma had disappeared. To what extent this was due to absorption, digestion, or other processes was not known. The rapid passage of spermatozoa and seminal fluid was thought to be due to muscular contraction of the uterus.

Joël (1942) prepared a monograph on the study of human spermatozoa. He describes the "spermiophagie" and shows photomicrographs of sperm being phagocytized.

B. Histology of Male Genital Tract

The earliest available record of research into the basic structure of the boar testicle is reported by Leydig (1850). Later Regaud (1901) carried the study further, giving more

details on morphological changes of spermatogenesis. Schmaltz (1911) describes the histological structure of the genital tract of the boar and summarizes the information available at that time. All of these workers make reference to the great number of interstitial cells in the boar testis. Boscom and Osterud (1927) studied the embryological development of the pig testis. After extensive histological study of boar testicles McKenzie, Miller, and Bauguess (1938) reported the following:

> The seminiferous tubules of the boar are similar to those of other mammals, but the interstitial tissue is somewhat more plentiful. In cross section, the ducts of the epididymis have a regular circular outline. The epithelium rests on a basement membrane, and is surrounded by circular, smooth muscle fibers. The lumen of the ductus epididymis is lined by a pseudo-stratified, columnar secretory epithelium. The brush-like projections on the free end of the cells are stereocilia, and presumably are non-motile. In the head region, the epithelium is tall; it is noticeably lower in the mid-region and still lower in the tail. However, the secretory activity, as judged by the presence of droplets on the stereocilia appears to be greatest in the tail region and least in the head of the epididymis. The body region shows a transitional stage between the head and tail.
>
> From the point where the convoluted ductus epididymis straightens into the ductus deferens, to the urethral orifice is a distance of 25 to 30 cm. Normally the lumen is round but on fixation the epithelium is thrown into folds due to contraction of the walls. The lumen is lined by pseudo-stratified columnar epithelium, somewhat lower than that of the epididymis. Stereocilia containing secretion droplets line the free ends of the epithelium. Stereocilia and secretion droplets on the epithelium are reduced or entirely absent in the urethral end of the vas deferens. They are prominent in the epididymal end however.

McKenzie et al. also present a complete histological description of the accessory genital glands (seminal vesicles, bulbourethral and prostate) and the urethra of the boar.

Phillips and Andrews (1936) reported on developmental changes of porcine testis as indicated in the following statement:

> Little development of the germinal epithelium was observed until after 84 days of age. The spermatogonia were arranged in a loose layer on the basement membranes at 12 days and the remainder of each tubule was filled with an opaque mass. In the succeeding stages the spermatogonia became arranged in a compact and orderly fashion around the basement membrane and primary spermatocytes gradually appeared, so that a few were present in all tubules at 84 days of age. In the 105 day stage secondary spermatocytes had appeared in some tubules, and at 126 days the epithelium appeared completely organized except for spermatozoa. Spermatozoa were present in some tubules at 147 days.
>
> It would seem from this series of animals that the testes of the boar develop slowly up to about 84 days of age and that after this age a more rapid development sets in, with spermatozoa first being produced at about 147 days.

Roosen-Runge (1952) compared the histological structure of rat, boar, and dog testes and reported that the boar showed essentially the same kinetic pattern of spermatogenic waves of the seminiferous tubules as rat testicle. However, there were a few minor fine points of difference.

Studies of the male genital tract of species other than porcine have been done by Hooker (1944), whose work was on the interstitial cells of the bull. Wislocki (1949) studied seasonal changes in the testis, epididymis, and seminal vesicles of deer on the basis of changes in lipids, glycogen,

acid and alkaline phosphatase, and PAS. Elftman (1950), who worked on mouse testicle, emphasizes the importance of the Sertoli cell in transferring material from the vascular supply to the lumen where maturation of spermatids takes place.

Lynch and Scott (1951) state that the distribution of the tubular lipid which appears to be in the Sertoli cells, in relation to the maturing sperm, is compatible with the hypothesis that the Sertoli cells have a nutritive function.

The distribution of lipids, glycogen, and phosphatases in the human testis has been reported by Montagna (1952a). Montagna (1952b) reported in man that there is a high lipid content in Sertoli cells as well as in spermatogonia and primary spermatocytes. Leblond and Clermont (1952) clarified and defined the stages of the cycle of the seminiferous epithelium in the rat. Oakberg (1956) describes spermiogenesis and the cycle of the seminiferous tubules of the mouse after the PAS technique. Burgos and Fawcett (1955) present some electron microscopic observations of the acrosome differentiation and the nature of the manchette in the cat testis. Pelc (1957) gives autoradiographic evidence that DNA (desoxyribonucleic acid) is synthesized from RNA (ribonucleic acid) in the mouse testis. Histochemical studies of glycogen content of testes of domestic animals was reported by Nicander (1957). He found that most of the glycogen in the tubules was in the Sertoli cells. There was no study included on the boar.

C. Histology of Female Genital Tract

The histological structure of the ovary, oviduct, uterus, cervix, and vagina of the sow was recorded by Schmaltz (1911). He illustrates many folds in the ampulla of the oviduct. He also described the histological structure of the sows uterus and ovary. Corner (1921) studied the cyclic changes in the ovaries and uterus of the sow and presented the following description of the sows uterus during estrus.

> During the days of oestrus the uterine epithelium has a total thickness of 25 to 30 micra. As shown in the figures, it is not obviously columnar, but presents an arrangement which is deceptively suggestive of stratification. The cells are so closely compressed together laterally, and at the same time have attained so low a form, that they are rather irregularly packed and the nuclei thus appear to be arranged in several layers. On careful study many of the cells appear to extend from base to surface of the epithelium, but others seem to be impeded by their neighbors from reaching the free surface. The cells are small, since there is a relatively low proportion of cytoplasm to nucleus; so that, taking all these criteria together, a histologist coming upon such a tissue for the first time might perhaps class it among epithelia like those of the urinary bladder rather than among the secretory types of epithelial tissues.
>
> Three details are especially to be noted at this stage: First, mitotic figures are very numerous in the epithelium, in some specimens occurring as often as one in every 50 nuclei. The epithelium, therefore, is actively proliferating. Second, a contrary process is also indicated by the presence here and there of phenomena of degeneration; at the base of the epithelium there are points where two or three cells have become vague of outline, with chromatolysis of the nuclei, so that a small vacuole is formed in which lie a few nondescript nuclear fragments or granules. This degeneration is, as we shall see, merely the latter stage of a phenomenon which sets in slightly earlier than the period of oestrus. The same may be said of

the third fact of especial interest, namely, the
presence, in large numbers, of neutrophilic poly-
morphonuclear leucocytes in the subepithelial con-
nective-tissue, and even of a few which are embedded
in the epithelium, presumably making their way into
the lumen.

During oestrus the stroma of the uterus, in mature
animals, is very edematous, so that the interspersed
cells are widely spaced.

There are a few mitoses in the cells of the super-
ficial gland tubules, but none in those of the deep
glands. A curious feature is the presence, in some of
the gland cells, of highly chromatic extra-nuclear
granules reaching a diameter of one to two micra. The
nuclei of cells possessing these granules are usually
of normal appearance; but I have tentatively considered
the granulation as a degeneration phenomenon affecting
a few cells of the glands. It is seen only during
the oestrous period.

Corner demonstrated no glycogen in the sow uterus using Best's

carmine and iodine method.

McKenzie (1926) studied the estrus cycle in the sow by

means of vaginal smears and histological studies. Green

(1950) describes the sow uterus in the first and second day

of heat:

The epithelium is composed of tall columnar
cells so tightly compressed that they are pseudo-
stratified. Underlying the epithelium there are
several layers of fibroblasts lying rather close
together. Among these fibroblasts there are a few
lymphocytes and polymorphs; these latter elements
are usually closely applied to the epithelium,
with an occasional polymorph penetrating the epi-
thelium.

The endometrial stroma is edematous, the edema
being most pronounced in the region just below the
epithelium. The coiling of the glands, though
present, is not pronounced. The glands of the sow
are always coiled, but the degree of coiling and the
length of the gland change during the cycle. Here
and there throughout the glands there are cells that
are degenerating.

In addition to basic morphological studies of uterine structure, much attention has been directed to specialized phenomena and techniques in the ruminant genital tract. Weber et al. (1948) studied the phenomena of metrorrhagia in the virgin heifer. Roark and Herman (1950) made an extensive investigation of histological changes associated with the estrus cycle. Weeth and Herman (1952) reported histochemical studies of the bovine uterus and oviduct. Foley and Reece (1953) studied histochemical reactions of the bovine uterus and placenta. With the exception of the work of Wislocki and Dempsey (1946) on histochemical reactions of the placenta in the pig, no histochemical studies have been made on the genital tract of the sow. Hatch (1940) studied the anatomical changes of the bovine uterus during pregnancy. Herrick (1950) studied the histological changes of the cervical mucosa of the cow during the estrous cycle. Hadek (1955) presented histochemical evidence of active secretion taking place in the oviduct of the ewe.

D. Artificial Insemination in Swine

1. Collection of boar semen

One of the earliest records of collection of boar semen is by McKenzie (1931). He used a simple type of artificial vagina, consisting of a long piece of thin rubber tubing, which is attached at one end to a large key ring and at the

other end to a collecting vessel. Pressure on the penis was maintained on the outside by hand. Milovanov (1932) and Rodolfo (1934) reported that semen could be obtained from the boar quite simply by means of a dummy sow and artificial vagina. Hutchings and Andrews (1945) collected semen from a large number of boars using an artificial vagina similar to that used on the bull, only smaller, in his work on culturing Brucella organisms from boar semen. Herrick (1949) collected semen from boars with a bovine artificial vagina in studying infertility in boars. Christensen (1953) used a phantom sow covered with sow hide with an artificial vagina mounted inside in his studies of impotentia coeundi. He found that it was easier for boars to mount the phantom than a real sow. Dziuk, et al. (1954) reported that boar semen can be collected by using electrical stimulation with a rectal probe. There was no record of a complete ejaculate being obtained by this method. Westerman[1] used a sow restrained in a crate built for that purpose. When the boar mounted, a small artificial vagina was used to collect the semen. Polge (1956) describes and illustrates a dummy sow and artificial vagina which has been used in England. Aamdal and Hogset (1957) describe and illustrate a dummy sow and artificial vagina which has been

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[1]Westerman, C. J. West Bend, Wisconsin. Information on operation of swine artificial insemination ring. 1956. (Private communication).

used extensively in Norway. Aamdal et al. (1958) illustrated some improvements of both dummy and artificial vagina which have been developed during continued use of this equipment in Norway.

2. Physiological studies of boar semen

McKenzie, Miller, and Bauguess (1938) studied whole and fractionated semen collections. Sperm-cell counts, sperm-cell morphology, volume of ejaculate, and duration of motility were reported. They found variations in volume of ejaculate, sperm-cell density, and duration of motility that seemed to be related to frequency of ejaculation. Moore (1946) studied the metabolism and motility of ram, boar, and stallion spermatozoa and came to the following conclusions:

> Both ram and boar spermatozoa were found to exhibit glycolytic activity. Some evidence was also obtained indicating a similarity between boar and stallion spermatozoa. The reducing substances in ram semen are converted to lactic acid but no conversion of the reducing substances in boar and stallion semen could be demonstrated.
> Glucose, fructose, and mannose are glycolytically converted to lactic acid by ram and boar spermatozoa. Lactic acid appears to be the sole product of sugar metabolism by these spermatozoa. Neither ram nor boar spermatozoa were able to utilize galactose or maltose.
> The results of the investigations with the above sugars, other sugars and related substances indicate the absence of enzymes in ram and probably in boar spermatozoa necessary to: (a) hydrolyze the alpha and beta glucosidic linkages of di- and polysaccharides, (b) to utilize sugars modified by oxidation of the aldo group or by substitution of an amino group for a hydroxyl group, and (c) to utilize sugars modified by variations in the configuration of glucose, other than those represented by fructose and mannose.

Glover and Mann (1954) studied 20 ejaculates from the same boar at weekly intervals. The total volume of ejaculates varied from 240 - 500 ml. and spermatozoa concentration from 50,000 to 177,000 per mm^3. Chemical analysis revealed 2.4 - 12.6 mg. per 100 ml. fructose, 5.9 - 23.1 mg. per 100 ml. ergothionine, and 32- 156 mg. per 100 ml. citric acid. Ejaculation was studied by the method of fractionated collection. The earliest to be emitted was the "pre-sperm fraction" from which spermatozoa and seminal vesicle secretion were absent. The next was the "sperm rich fraction" which showed not only a high sperm-cell content but contained also substantial quantities of substances derived from the seminal vesicles (fructose, ergothionine, and citric acid). The "post-sperm fraction" consisted mainly of accessory gland secretions.

Glover (1955) has this to say about chemical constituents of semen:

> The normal sugar of semen in all domestic animals is fructose, and it is derived from blood glucose. Fructose can be utilized by the spermatozoa as a substrate for motility both in the presence and absence of oxygen. But in the presence of a small but adequate supply of oxygen, the spermatozoa may oxidize lactic acid, which is the main product of fructolysis.
>
> Apart from fructose and lactic acid, the spermatozoa are able to utilize a number of other substances such as glucose, mannose, and various fatty acids. Some of these substances may be present in the secretions of the female reproductive tract. This would account for the fact that spermatozoa, even if deprived of seminal plasma, can survive and retain their fertility when placed in the uterus. On the other hand, it must also be remembered that while in the absence of oxygen the spermatozoa cannot remain motile without a

glycolysable sugar (fructose); aerobically, that is
in the presence of oxygen, as for example, when in
the female tract, the spermatozoa can utilize their
own intracellular reserve (probably a lipid), and
survive at the expense of the metabolic energy de-
rived from the oxidation of this intracellular
source.

Roy (1955) studied the storage of boar and stallion sper-
matozoa in glycine-egg-yolk medium. Johari (1956) studied
the gel mass in the semen of the boar.

3. Artificial insemination of sows

Rodin and Lipatov (1935) reported a technique of arti-
ficial insemination of sows. Anderson (1945) gives a tech-
nique for inseminating sows using a 50 ml. glass syringe, a
piece of rubber pressure tubing 45 cm. long and about 4 mm.
inside diameter. Ito, Niwa, and Kudo (1948) showed that arti-
ficial impregnation of sows can occur with a more concentrated
spermatozoa suspension and the elimination of much of the
natural fluid. Polge (1956) illustrates a technique of in-
semination of sows with a rubber tubing pipette and a pressure
bottle and pump. Aamdal and Hogset (1957) illustrated and
described an inseminating pipette consisting of a plastic tube
about 50 cm. long with a diameter of 7 - 8 mm. About 2 cm.
from the tip, a plastic balloon (diameter 4 cm.) is fixed to
block the cervical canal. The balloon is inflated by means
of a pump and a small piece of polyethylene tubing. A dual
purpose 150 ml. plastic bottle, used for transportation of

semen, is coupled to the inseminating pipette held upside down and collapsed by hand to force the semen through the pipette into the sow's uterus.

III. MATERIALS AND METHODS

A. Collection of Semen

1. <u>Dummy</u>

A phantom sow was constructed according to measurements taken from actual sows. The framework was constructed of oak lumber. The plans for this are shown in Figure 1. Narrow wooden strips were used to form the curved contour of the sows back. After this was done, the surface was covered with canvas, which was attached by means of glue. Latex mold material was applied to the surface to produce a tough skin-like covering. Three coats were applied at 24 hour intervals. The legs were constructed of two inch and two and one half inch metal pipe material and were made adjustable for different size boars. The dummy was mounted onto a three quarters inch plywood board. Cleats were affixed to the board to provide traction when the boar mounts. Holes 5 inches in diameter were placed in the sides of the phantom between sections 4 and 5 and sections 5 and 6 (Figure 1), to allow for manipulation of the vagina and collecting vessel during operation. The finished dummy is shown in Figures 2, 3, and 6.

2. <u>Artificial vagina</u>

The most satisfactory results were obtained with an artificial vagina constructed of an 8 inch length of radiator hose

with an inside diameter of one inch. Three quarter inch or one inch Gooch tubing is used as the innerliner. If a 9 or 10 inch length is used, it allows for a portion to be deflected over each end of the radiator hose. A half inch hole is cut through the radiator hose 4 inches from one end to permit the introduction of warm water into the outer cavity of the vagina. A valve-like retaining mechanism is produced by placing a short piece of one inch Gooch tubing over the hole. The outer space of the vagina is filled with water 130 degrees Fahrenheit; when the rubber material is warmed the resulting temperature is approximately body temperature of a sow, 102 - 104 degrees Fahrenheit. The vagina may be either held during use or suspended in the dummy by means of heavy rubber bands which pass around the vagina at each end and attach to hooks fixed inside the phantom. The separate parts of the artificial vagina are shown in Figure 4. Figure 5 shows the artificial vagina assembled and ready for use.

Little difficulty is encountered in training boars to mount the dummy. The boars were used on an estrous sow for several days after which time, the dummy was substituted for the sow. Figure 6 shows the dummy with artificial vagina suspended in it ready for use. The boar serving the artificial vagina is shown in Figure 7.

B. Methods of Studying Semen

Several methods were used to collect physiological data for the semen samples collected.

The pH was determined by means of a Beckman pH meter.

The specific gravity was studied with a hydrometer covering a range of 1.000 to 1.120.

The motility was graded (1, 2, 3, 4, or 5) according to the system outlined by Asdell (1955). This system is in general use in bovine artificial insemination establishments and is outlined as follows:

1. All spermatozoa non-motile

2. Weak oscullatory motion, large numbers non-motile

3. About equal proportions with progressive and oscullatory motion: 25 per cent non-motile

4. Most with progressive motion

5. Nearly all with high degree of progressive motion

Sperm-cell density was determined by the method outlined by Laing (1955) by using a regular blood hemocytometer and making a direct count.

To study the percentage of live and dead sperm, the technique of Lasley, Easley, and McKenzie (1944) was used.

Samples of semen were collected from boars which were maintained for experimental work. Motile spermatozoa were present in all samples used. Smears were made from fresh semen immediately after collection and also from semen which

had been stored for varying periods of time under different conditions. Control smears were made of spermatozoa which were washed by repeated centrifuging, using a Ringer-phosphate solution, buffered at pH 7.2.

The following staining procedures were used:

1. Bodian's protargol method. After preliminary drying in air, the smears were fixed in Bodian's fixative No. 2 (80 per cent ethyl alcohol 90 ml., formaldehyde 5 ml., and glacial acetic acid 5 ml.). Staining was carried out according to Bodian's method as modified by Dawson and Barnett (1944). The smears were dehydrated, cleared, and covered in clarite.

2. Gomori's reticulum stain. After drying in air, the smears were fixed for 24 hours in 10 per cent formalin. Staining was carried out according to Gomori's reticulum stain (Mallory, 1938, p. 164).

3. Glycogen. Smears were fixed for 24 hours in chilled Rossman's fluid (90 ml. of absolute alcohol saturated with picric acid and 10 ml. of 37 per cent formaldehyde). After fixation, the smears were washed in absolute alcohol followed by distilled water before staining with the Bauer-Feulgen method (Bensley and Bensley, 1938, p. 105). Control smears were treated with saliva or diastase for one hour at 37 degrees Centigrade before staining.

4. Feulgen reaction. Smears were fixed 12 to 24 hours in 10 per cent formalin and stained by means of the Feulgen reaction of Stefano (1948) for desoxyribonucleic acid.

5. Periodic acid-Schiff procedure. Smears were fixed for 24 hours in Zenker's fixative. Following fixation the smears were washed in distilled water, stained by the PAS procedure according to McManus (1946) and then dehydrated, cleared, and mounted in synthetic resin. In order to remove polysaccharides such as glycogen, control smears were incubated in diastase one hour at 37 degrees Centigrade before treatment.

6. Alkaline phosphatase. Smears were fixed for 12 hours in 80 per cent ethyl alcohol at approximately 4 degrees Centigrade. This reaction was carried out at pH 9.4 for 4, 8, 24, 48, and 72 hours. According to Gomori (1952, p. 184) sodium glycerophosphate was used as a substrate.

7. Acid phosphatase. After fixation in chilled acetone for 4 hours, the smears were incubated in a sodium glycerophosphate substrate at pH 5.0 for 48 and 72 hours at 37 degrees Centigrade as outlined by Gomori (1952, p. 193).

8. Gram's stain. Hucker's modification of Gram's method as described by Guyer (1936) was used.

9. Baker's acid hematin test for phospholipid. This procedure and the associated control (or pyridine extraction) test were carried out as prescribed by Baker (1946). After

preliminary drying, the smears for hematin treatment were fixed in formol calcium and the control smears in Baker's "weak Bouin's fluid".

10. Casarrett stain. After preliminary drying, smears were fixed in 50 per cent ethyl alcohol and 50 per cent ether solution. The smears were stained according to Casarrett (1953) at 40 degrees Centigrade, washed in distilled water, dehydrated, cleared, and mounted in synthetic resin.

11. Aceto-carmine stain. After drying, smears were fixed and stained simultaneously in aceto-carmine solution as recommended by Green (1950).

12. Sudan black B. Smears were fixed in 10 per cent formalin for 6 hours. Following fixation, they were stained in a saturated solution of sudan black B in 70 per cent alcohol for times varying from one to two hours. After staining, the slides were dipped into 70 per cent ethyl alcohol, washed in distilled water, and mounted in Farrant's medium.

C. Insemination of Sows

A preliminary breeding experiment was carried out on 4 gilts. The gilts, 8 months of age, were tested twice daily by putting a boar into the pen with them. Estrus could be observed by swelling and hyperemia of the vulva and was also indicated by the boar's interest. During the first day of heat, the gilts would not stand for the boar to mount. On

the second day they would stand. The gilts were inseminated
on the second day of heat using 100 ml. of fresh whole semen.

Due to the fact that the gilts and boars were in the same
building, it was possible to accomplish the insemination with-
in 15 minutes after the semen was collected. The equipment
used for this is shown in Figures 8 and 9. The sows were
restrained in a modified farrowing crate so that insemination
could be carried out with as little difficulty as possible.
A pressure bottle (Figure 11), as used by Polge (1956) in
England, was used with a modified bovine inseminating pipette.
A regular disposable plastic bovine inseminating pipette was
gently heated over a flame until the area three inches
from one end became soft. The pipette was bent 30 degrees
at this point and allowed to harden. The other end was
treated similarly; only one inch of the end was bent 30 de-
grees. The end with the short bend was inserted into the
vagina of the sow for insemination. The off-set angle at both
ends of the pipette facilitated manipulation of the tip of
the pipette past the nodular projections in the sow's cervix.

A 50 ml. glass syringe with a rubber adapter was also
used for insemination with a pipette (Figure 10). It is not
as satisfactory as the pressure bottle.

A later experiment was done on 40 sows at the Iowa State
College Swine Breeding Farm at Napier. These sows were in-
seminated with from 50-200 ml. of fresh whole semen within

two hours after collection. Some of the sows were restrained
in a chute during insemination; others were inseminated in an
open pen with no restraint.

D. Histological Material

1. Sows

Five sows were used to determine the transformations and
fate of the spermatozoa after their deposition in the female
genital tract. These sows were bred naturally on the second
day of heat and were then slaughtered at varying periods of
time following breeding. Table 1 is a description of the
sows and the details of material collected.

At the appropriate time after breeding, the sows were
stunned and bled from the anterior vena cava. The abdomen

Table 1. Description of sows and details of material collected

Sow no.	Age in months	Breeding history	Time slaughtered after breeding
Sow 1	16	Farrowed three months previously	1 3/4 hr.
Sow 2	16½	Cesarean three months previously	27 hr.
Sow 3	16 3/4	Farrowed three and one half months previously	30 min.
Sow 4	5		10 min.
Sow 5	5		8½ hr.

was opened and the entire genital tract was removed as quickly as possible. The oviducts were clamped off with forceps and the uterine material was collected from various areas.

Sections of uterine tissue were taken from the right uterine horn at three different points (Figure 12); section one near the bifurcation, section two in the middle of the horn, and section three near the oviduct end of the horn. The left uterine horn was opened and the contents removed. Smears were made of this material to compare with freshly ejaculated semen smears. When no fluid was present in the uterine lumen, smears were made by gently pressing the slide against the exposed uterine mucosa.

Three sections of the right oviduct were collected for histological study (Figure 12); section one near the ostium uterinum, section two in the middle of the oviduct, and section three in the ampulla of the oviduct near the termination of the infundibulum.

The left oviduct was divided into 4 segments. These were canulated and flushed out with ringer-lock solution into a watch glass. The few drops of saline which came through each segment were examined microscopically for the presence of spermatozoa.

Three samples of the right ovary were taken from posterior to anterior as shown in Figure 12.

The cervix was examined grossly for the presence of a mucous plug.

2. Boars

Five boars, 16 months of age, were used to study the development of spermatozoa in the testicle and the changes that transpire during maturation in the epididymis. Repeated semen samples were analysed as a preliminary study to determine the sperm-cell count and pH of the semen of these boars. Smears were also made for staining. After these studies were completed, the boars were restrained in lateral recumbency and castrated, removing as much of the vas deferens as possible. The right testicle was used for histological study and the left testicle was used for gross studies and to make smears of epididymal fluid and spermatozoa for comparison with ejaculated sperm.

Figure 13 shows the areas which were studied histologically. Three specimens were taken from the testicle; number one from the anterior part, number two from the middle, and number three from the posterior part of the testicle. Three specimens were collected from the head, body, and tail of the epididymis. Three sections of vas deferens were taken for fixation; number one close to the tail of the epididymis, number two from the part of the vas deferens related to the

head of the epididymis, and number three from the area
farthest removed from the testicle and epididymis.

E. Histological Methods

1. Fixation and embedding

Six different fixation fluids were used:

1. 10 per cent neutral formalin

2. Zenker-formol

3. Acetic acid 5 ml., 40 per cent aqueous formalde-
    hyde solution 5 ml., 80 per cent ethyl alcohol
    90 ml.

4. 80 per cent alcohol

5. Acetone

6. Rossman's

The 10 per cent solution of neutral formalin (equal to
4 per cent formaldehyde) was used as a general fixative. The
tissue samples were allowed to remain in the formalin fixative
for 24 to 48 hours. After the fixation period, the specimens
were dehydrated in butyl alcohol and embedded in Altmann's
mixture.

Zenker-formol fixative was used for the tissue to be
studied by the PAS technique. Samples of tissue were allowed
to remain in the fixative for 24 to 48 hours. Then they were
washed, dehydrated in butyl alcohol, and embedded in Altmann's
mixture.

Tissue to be treated with Bodian's protargol technique was fixed for 48 hours in the alcohol, formalin, and acetic acid mixture. After fixation, the tissues were dehydrated through butyl alcohol and embedded in Altmann's mixture.

The tissue samples to be studied for alkaline phosphatase were fixed in 80 per cent ethyl alcohol for 24 hours at two degrees Centigrade. After fixation, the tissue was dehydrated in absolute alcohol, cleared in cedar oil, and embedded in Altmann's mixture by infiltration through benzene.

The tissue samples to be studied for acid phosphatase were fixed in acetone for 24 hours at two degrees Centigrade. After fixation, the specimens were embedded in a manner identical to the alcohol fixed tissue.

The tissues to be studied by means of the Bauer-Feulgen technique for glycogen were fixed in Rossman's fluid (90 ml. of absolute alcohol saturated with picric acid and 10 ml. of 37 per cent formaldehyde) in a refrigerator at two degrees Centigrade for 24 hours. After fixation, the tissues were dehydrated and embedded in a manner identical to the alcohol fixed tissues.

2. Sectioning and mounting

Sections of the paraffin blocks were cut at 8-12 micra in thickness with a rotary microtome. Serial sections were made, using from 5-10 sections on each slide and making 5

slides from each paraffin block. The paraffin sections were affixed to the glass slides by means of Mayer's albumin-glycerine mixture.

3. <u>Staining techniques - histological</u>

(a) <u>Hematoxylin and eosin.</u> Sections were routinely stained with Harris' alum hematoxylin, Mallory (1938, p. 72), and precipitated eosin Y technique, according to Pearson (1942). This technique was used after fixation in 10 per cent neutral formalin, Rossman's, Zenker-formol, alcohol, and acetone.

(b) <u>Weigerts, Van Gieson's and Heidenhains.</u> The following three staining techniques were used in succession: Van Gieson's acid fuchsin and picric acid stain for collagenous fibers (Guyer, 1936, p. 250); Weigerts stain for elastic fibers (Mallory, 1938, pp. 168-169); Heidenhain's iron hematoxylin for nuclear detail (Mallory, 1938, pp. 74-75). This technique was used after fixation in Rossman's fixative, Zenker-formol, and alcohol.

(c) <u>Silver impregnation.</u> For demonstration of reticular fibers, Gomori's reticulum stain was used (Mallory 1938, p. 164), using ammoniacal silver solution. This technique was used following fixation in Rossman's fixative, alcohol, and acetone.

(d) <u>Tri-chrome stain.</u> Following fixation in Zenker-formol and formalin, acetic acid and alcohol mixture, sections were stained by means of the Crossman (1937) modification of Mallory's connective tissue stain.

(e) <u>Bodian's protargol.</u> After fixation for 48 hours in the alcohol, formalin, and acetic acid mixture, the sections were prepared by the method of Dawson and Barnett (1944) for Bodian's protargol.

4. <u>Staining techniques - histochemical</u>

(a) <u>Alkaline phosphatase.</u> For the histochemical demonstration of alkaline phosphatase, the method of Gomori (1952, p. 184) was followed. After fixation in 80 per cent alcohol, the tissues were embedded in paraffin by means of benzene infiltration. The sections were deparaffinized in xylol and were incubated for 4 hours in a calcium solution containing sodium glycerophosphate and magnesium ions at pH of 9.4. Control sections were prepared by omitting the substrate, sodium glycerophosphate, in the incubating solution. The sites of phosphatase activity were made visible microscopically by converting the precipitated phosphate into visible brown sulfides.

(b) <u>Acid phosphatase.</u> For the demonstration of acid phosphatase, the method of Gomori (1952, p. 193) was used.

After fixation in acetone, the tissues were embedded in paraffin. After deparaffinization, the sections were incubated for 24-48 hours in anacetate buffer solution at pH 5, containing lead nitrate and sodium glycerophosphate. Tetrachlorethylene was used for clearing before mounting in synthetic resin. Control sections were incubated in a buffer solution without the sodium glycerophosphate.

(c) Periodic acid-Schiff reaction. For the localization of periodic acid reactive carbohydrates, tissues were fixed in Zenker's formol solution for 24-38 hours. After embedding and sectioning, the material was treated with the PAS technique as developed by McManus (1946). Control slides were incubated in diastase for one hour at 37 degrees Centigrade in order to remove glycogen. The sections were counter stained with saturated picric acid solution.

(d) Glycogen technique. For the demonstration of glycogen, the tissue was fixed in Rossman's fluid at two degrees Centigrade, cleared in cedar oil, and embedded in paraffin. The sections were cut at 8-12 micra and stained with the Feulgen-Bauer technique (Bensley, 1938, p. 105). Control sections from which the glycogen had been digested in saliva or diastase were stained simultaneously.

(e) <u>Sudan black B</u>. For the localization of phospholipid material, sudan black B was employed following fixation in 10 per cent formalin.

(f) <u>Oil red O</u>. Cytoplasmic inclusions of lipid droplets were demonstrated by means of oil red O technique according to Bell[1] following fixation in calcium cadmium formol as described by Baker (1944). Sectioning was done with the freezing microtome. Following staining the cover slips were mounted with Farrant's mounting medium.

(g) <u>Ribonucleic acid</u>. For the differentiation of ribonucleic acid, the methyl green and pyronine mixture of Unna and Pappenheim (1910, p. 584) was used following alcohol, acetic acid, and formalin fixation. Control sections were incubated in a 0.01 per cent solution of ribonuclease at 60 degrees Centigrade for one hour.

(h) <u>Desoxyribonucleic acid</u>. For the localization of desoxyribonucleic acid, tissue sections from material fixed in formalin were treated with Feulgen's reaction as discussed by Stephano (1948) following hydrolysis for 6-10 minutes in N HCl.

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[1]Bell, J. T. Ames, Iowa. Oil red O technique for staining lipids. 1958. (Private communication.)

IV. OBSERVATIONS

A. Cytomorphosis of Boar Spermatozoa

1. Acid phosphatase

In the seminiferous tubules, the early spermatids are lightly colored after 48 hours incubation (Figure 38). The acrosomic granule and the developing acrosome show up as darker structures. When the spermatid reaches that stage of spermiogenesis where the head is directed toward the basement membrane and embedded in the Sertoli cell cytoplasm, it is surrounded by a very darkly reacting area. It is difficult to ascertain whether the reaction is in the spermatid itself or in the Sertoli cell cytoplasm.

After the definitive spermatozoa are released from the Sertoli cells and are free in the lumen, many of them show a marked reaction for acid phosphatase involving the entire sperm-cell. Others show the reaction to a lesser extent, with the head cap reacting most distinctly.

Spermatozoa smears made from material taken from the epididymis show a variety of reactions, ranging from no reaction to reaction of the entire sperm-cell. Between these extremes, some spermatozoa showed the head cap reacting more distinctly than the postnuclear cap. Others showed the posterior nuclear cap most distinctly. In sections of the epididymis the spermatozoa reacted quite distinctly when

massed together. Isolated individual spermatozoa seemed to be uniformly darkened, including the protoplasmic droplet.

Smears made from ejaculated semen showed a faint reaction, most predominantly in the postnuclear cap (Figure 21), however, some showed the most distinct reaction in the acrosomal area (Figure 20). The reaction was most distinct in smears made from fresh semen. In semen stored for 120 hours, no reaction was evident. Smears prepared from spermatozoa which had been washed free of seminal plasma by repeated centrifugation were non-reactive. Areas of dried seminal plasma on the slide reacted quite strongly (Figures 20 and 21).

In the female genital tract, the spermatozoa did not react to the acid phosphatase treatment. The fluid present in the uterine horn reacted uniformly on the smear and the spermatozoa stood out as clear non-reactive areas. The leucocytes which were present on the smears were very reactive.

2. Alkaline phosphatase

In the seminiferous tubule, the developing acrosome of the early spermatids reacts very distinctly to the technique for demonstration of alkaline phosphatase activity. This is more intense in the later spermatids which are intimately related to the Sertoli cells. After the definitive spermatozoa

become detached and are free in the lumen of the tubule, they react most distinctly in the acrosomal area.

In the epididymis the acrosomal area is most reactive. The posterior part of the head, middle piece, protoplasmic droplet, and tail react to a lesser degree. Smears made from epididymal fluid contained spermatozoa which did not react as intensely as those in the sections of the epididymis. Spermatozoa observed in sections of the vas deferens were very faintly reactive, showing very little difference between acrosome and other parts of the sperm-cell.

Spermatozoa observed on smears made from ejaculated semen reacted very distinctly in the acrosomal area (Figure 23). This reaction was much more distinct than that observed for acid phosphatase. Spermatozoa in smears, made from semen stored over 72 hours, showed a very faint reaction or no reaction at all. In smears made from freshly ejaculated semen, it appeared that the reaction was most distinct in areas on the smear where there was an accumulation of dried seminal plasma. These granular aggregations reacted quite strongly. Smears prepared from spermatozoa which had been separated from the seminal plasma by repeated washing and centrifuging reacted very faintly or not at all.

Spermatozoa observed on smears made from the uterus following service by a boar showed very little to no reaction to

the alkaline phosphatase technique. The leucocytes present in the smears were quite reactive.

3. Sudan black B

In the seminiferous tubules, the middle piece of the late spermatids shows a positive reaction for phospholipid. Mature spermatozoa which are free in the lumen show the same reaction.

Epididymal spermatozoa, ejaculated spermatozoa, and those in the female genital tract show a similar reaction (Figure 24).

4. Gomori's silver

In the seminiferous tubules, the heads of the spermatozoa are uniformly stained a dark purple. Spermatozoa in the epididymis stained the same.

In smears of epididymal spermatozoa, the postnuclear cap showed the most impregnation. In some instances where a sperm-cell tail crossed the head of another sperm-cell, two lines of silver impregnation appeared on either side of the tail filament. This gave the appearance of lines in the equatorial segment region of the head.

In ejaculated semen smears, the posterior head cap was most definitely impregnated (Figure 25). Most spermatozoa smears showed the same reaction, regardless of storage time.

One smear made after 98 hours storage showed uniform impregnation throughout the heads.

5. PAS

Sections of testicular tissue treated with the periodic acid-Schiff reaction show spermatids in all stages of spermiogenesis, from the very early spermatid to the definitive sperm-cell. According to the classification of Leblond and Clermont (1952), this sequence of changes can be divided into 4 phases; Golgi, cap, acrosome, and maturation. Figure 14 shows these 4 phases and the 17 different stages of spermiogenesis as it occurs in the boar.

The following is a description of the 4 phases of spermiogenesis:

1. Golgi phase. The first appearance of the spermatids follows the division of the secondary spermatocyte. These early spermatids are circular cells with a centrally located nucleus. The first appearance of the acrosomic granules are a few bright red granules which appear at one side of the nucleus. These granules coalesce into one large granule. The acrosomic vesicle develops around this granule, marking the end of the Golgi phase (Figure 15).

2. Cap phase. The head cap grows out of the acrosomic granule. A membrane protrudes out of the granule and spreads over the surface of the nucleus (Figure 16).

3. Acrosome phase. During this phase the head of the sperm-cell becomes differentiated and the manchette develops and the cell begins to resemble a spermatozoon (Figure 18).

4. Maturation phase. The excess cytoplasm is extruded (Figures 17 and 18) except for the protoplasmic droplet and the mature sperm-cell is released from the Sertoli cell into the lumen of the tubule.

6. **Tri-chrome stain**

In all smears prepared with this technique, spermatozoa stained the same regardless of origin or age of the material. The heads stained blue with the postnuclear cap being slightly darker than the acrosome. The tail and middle piece stained a bright red (Figure 27).

7. **Feulgen reaction**

In the early spermatids DNA granules are loosely distributed throughout the nucleus. As spermiogenesis progresses, the granules seem to condense into a homogeneous mass contained within the head of the sperm-cell. The body and tail do not react (Figure 28). The posterior margin of the head stains more intensely than the rest of the head.

8. **Methyl green and pyronine**

The cytoplasm of the early spermatids contains considerable ribonucleic acid as revealed by the staining technique.

As the spermatids undergo spermiogenesis, the RNA is extruded with the cytoplasm as residual bodies. No RNA was observed in the mature sperm-cells.

9. Casarrett stain

With the Casarrett staining technique, the spermatozoa are stained a pink color. The posterior part of the head stains more deeply than other parts of the sperm-cell (Figure 29).

10. Baker's hematin

The body or middle piece reacts most intensely. The posterior head cap reacts somewhat but not as much as the body (Figure 30). Control slides prepared by pyridine extraction do not show this reaction (Figure 31).

11. Aceto-carmine

Aceto-carmine staining differentiates the acrosome, equatorial segment, and posterior head cap. The acrosome is a light pink; the equatorial segment, a darker pink; and the posterior head cap, a deep red color. The body and tail are stained about as intensely as the equatorial segment (Figure 32). This technique revealed the most detailed sperm-cell morphology and was the easiest to use.

12. Gram's stain

Some of the preparations stain only the head; others the entire sperm (Figure 33). The results obtained with this technique were extremely variable.

13. Hematoxylin and eosin

Smears prepared with the hematoxylin and eosin technique show very few details of the sperm-cell structure. Figure 34 shows spermatozoa stained with hematoxylin and eosin.

14. Phase microscope studies

Morphological changes in the spermatozoa could best be followed by phase microscopy. After 24-48 hours of storage, the galea capitis became detached or damaged in many of the sperm-cells. Difficulty was encountered in taking photo-micrographs of living spermatozoa on the phase microscope. The movement of the spermatozoa made it impossible to get a clear picture. Figure 35 shows two sperm-cells which were fixed by mixing freshly collected semen half and half with 10 per cent formalin. The sperm were immobilized but much of the detail in the head region is lost.

15. Protargol

In the seminiferous tubules, the heads of the developing spermatids appear brown where they are embedded in the Sertoli

cell cytoplasm. Mature spermatozoa which are free in the lumen appear to be uniformly stained.

The spermatozoa, observed in the lumen of epididymal tubule sections, were uniformly darkened by the protargol treatment. Spermatozoa in smears made from epididymal fluid reacted most distinctly in the posterior head cap area. In smears made from ejaculated semen, the majority of spermatozoa reacted most distinctly in the posterior head cap area (Figure 37). This was quite regular, regardless of time after ejaculation. Occasionally the reaction was strongest in the acrosomal area (Figure 36).

16. Glycogen

Around the clusters of spermatid heads embedded in the cytoplasm of the Sertoli cells, there are a few fine granules of glycogen. After the definitive spermatozoa are released into the lumen, there is no evidence of glycogen in them.

In the epididymis the spermatozoa showed no evidence of glycogen, however, the cytoplasm of epididymal epithelial cells contained many glycogen granules.

Spermatozoa in smears of epididymal fluid were negative for glycogen.

All ejaculated spermatozoa were negative for glycogen by either the Bauer-Feulgen or PAS technique.

B. Histological Studies of Reproductive Tract of Boar

1. Testicle

(a) General histological observations. The parenchyma of the boar testicle is composed of the coiled and convoluted seminiferous tubules and interstitial cells. It is divided into lobules by connective tissue septa. There are a great number of interstitial cells of Leydig (1850) (Figure 44).

On high power examination of sections of the seminiferous tubules, it may be observed that different tubules are in different stages of spermatogenesis and spermiogenesis. The different stages can be identified on the basis of the morphology of the spermatids (Figures 40, 41, and 42). A great similarity was noted between the stages observed in boar testicles and the 14 stages of the cycle of the seminiferous epithelium in the rat as described by Leblond and Clermont (1952b).

The connective tissue septa of the boar testis contains many vascular elements. A profuse capillary network is interwoven among the interstitial cells. The seminiferous epithelium is supported by a basement membrane, composed of reticular fibers which are closely related to the capillary network. The seminiferous tubules varied from 200 to 300 micra in diameter.

The basal layer of the seminiferous epithelium is made up of spermatogonia which are found in various stages of

mitosis. The spermatogonia divide to become two primary spermatocytes. These may also be observed in stages of preparation for division as described by Leblond and Clermont (1952b). Following these changes, the primary spermatocytes undergo a rapid division to secondary spermatocytes. This is followed by another rapid division to form spermatids. The cells then undergo a series of changes, spermiogenesis, as described in Section A of Observations. The spermatids are closely related to the Sertoli cells during the last part of their development. At some stages of the seminiferous cycle, the lumen of the tubule is filled with free spermatozoa.

(b) <u>Acid phosphatase</u>. After 24-48 hours incubation, the strongest reaction is evident in the cytoplasm of the Sertoli cells in which heads of spermatids are embedded. Also the basement membrane of the seminiferous tubules shows a strong reaction (Figure 38). The spermatogonia, spermatocytes, and interstitial cells show a much fainter reaction.

(c) <u>Alkaline phosphatase</u>. After 4 hours incubation with the alkaline phosphatase technique, the most outstanding reaction is in the interstitial cells and the basement membrane of the seminiferous tubules. Capillary walls and the cytoplasm of the Sertoli cells, where spermatids are embedded, show a definite reaction but not as strong as the interstitial

cells and basement membrane (Figure 39). The nuclei of spermatogonia and spermatocytes show a slight reaction.

(d) <u>Glycogen (Bauer-Feulgen method)</u>. Glycogen granules can be easily demonstrated in the walls of the blood vessels and capillaries, which are woven among the interstitial cells and related to the basement membrane. Also glycogen was present in the cytoplasm of the Sertoli cells (Figure 43); particularly those related to late maturing spermatids. A few glycogen granules were observed in the cytoplasm of a small percentage of the Leydig cells. The glycogen granules were not observed in control sections incubated in saliva or diastase for one hour at 38 degrees Centigrade.

(e) <u>Periodic acid-Schiff reaction</u>. In the PAS preparations of boar testis, in addition to the PAS positive areas, glycogen granules are present as with the Bauer-Feulgen method. On slides incubated in saliva or diastase for one hour at 38 degrees Centigrade, the glycogen has been removed and only the PAS positive areas are evident. The acrosomic granule and the acrosome of developing spermatids stand out in bright red, in contrast to the surrounding material which is light pink. Also the reticular fibers of the basement membrane, capillaries, and blood vessels react positively. Circular or spherical empty vacuoles are very evident near the basement membrane in these preparations (Figures 42 and 49).

(f) <u>Oil red O lipid stain</u>. In sections prepared by frozen sectioning and staining in oil red O, bright red droplets of lipid material appear in the seminiferous tubules close to the basement membrane in the location where the empty vacuoles were noted in slides prepared through fat solvents (Figures 44, 45 and 96). The cytoplasm of the interstitial cells was filled with an orange stain (Figure 96). On oil immersion observation, they appeared to be numerous very fine lipid droplets dispersed throughout the cytoplasm.

(g) <u>Sudan black B</u>. In preparations treated with sudan black B, the most obvious reaction was in the cytoplasm of the Sertoli cells. These cells contained many dark staining granules or droplets in their cytoplasm, which made them stand out against the grey background of the other cells in the section. The nuclei of the Leydig cells were light colored, showing no reaction for phospholipid. Vacuoles were present in the basal area of the seminiferous tubules. These preparations were treated with xylol, so that any neutral fats or lipid material would have been removed. The erythrocytes in the capillaries showed a medium reaction to sudan black B, but were not as black as the Leydig cells.

(h) <u>Gomori's silver impregnation</u>. In slides prepared with silver impregnation, a very fine network of reticular fibers can be observed, forming the basement membrane of the

seminiferous tubules and a supporting network for the inter-stitial cells (Figure 46). The reticular fibers are continuous with collagenous fibers in areas of connective tissue concentration, like the septa. Many reticular fibers are present in capillary and blood vessel walls. The nuclear elements of the cells of the seminiferous tubules are colored black. Individual chromosomes in mitotic and meiotic divisions can be observed (Figure 47). In late spermatids or definitive sperm-cells, the nuclear material is condensed and appears as a uniformly dark purple, almost black, sperm-cell head.

(i) <u>Methyl green pyronine</u>. Slides prepared with methyl green pyronine technique for the demonstration of ribonucleic acid show areas of RNA concentration by red staining. In the seminiferous tubules, which have late spermatids, very distinct granules of RNA are present in the lumen of the tubules, as residual bodies. These appear to be concentrations of RNA which have been extruded from the developing spermatid with the cytoplasm during spermiogenesis. Control slides treated with ribonuclease did not contain these granules.

2. Epididymis

(a) <u>General histological observations</u>. The epididymis is composed of a coiled convoluted tubule, which is held together by a connective tissue supporting structure. Blood

vessels are abundantly mixed in among the twisted convolutions of the tubule. The tubule sections vary in size and shape in the different parts of the epididymis. In the head, the tubule sections are circular or oval, 450-550 micra in diameter with the epithelium being 70-80 micra in thickness. Many vacuoles are present in the basal parts of the epididymal epithelial cells (Figure 52). The body of the epididymis has circular tubule sections, which are 350-470 micra in diameter with the epithelium being 60-70 micra in height. In the tail, the epididymis tubule sections are 520-650 micra in diameter with an epithelium height of 80-240 micra. The basement membrane in the tail of the epididymis is circular but the lumen is stellate or undulating, due to variation in the thickness of the lining epithelium. The epithelium rests on a connective tissue basement membrane. The epithelial cells vary somewhat in the different parts of the epididymis. In the head, they are simple columnar or pseudostratified columnar cells with stereocilia appendages extending into the lumen. The nuclei are basally located for the most part.

In the body of the epididymis, there are simple columnar epithelial cells with stereocilia, which show evidence of secretory droplets. The nuclei are contained in the half of the cell nearest the basement membrane (Figure 53). Many vacuoles are present in the basal cytoplasm of the cells.

The cells of the tail of the epididymis are simple columnar, which vary considerably in height. The stereocilia are present on the luminal surface. The nuclei are arranged in a central zone across the epithelial layer at a regular distance from the surface, so that the nuclei form a dark band which is roughly parallel with the margin of the lumen.

There is a great amount of variation in cell thickness in the epididymal epithelium. This is probably related to secretion activity. The connective tissue increases around the tubule in the tail of the epididymis and gradually blends in with the vas deferens (Figure 58), where the tunic becomes very thick and muscular.

(b) <u>Acid phosphatase.</u> In sections of epididymis incubated for 24-48 hours with the acid phosphatase technique, a marked reaction was observed on the surface of the epithelial cells, including the stereocilia (Figure 54). This reaction extended into the cytoplasm nearest the lumen. The basement membrane and associated capillaries reacted, but not as strongly as the surface of the epithelium. The reactions were similar in all three areas of the epididymis. The vacuoles which appear in some areas in the basal cytoplasm of the epithelial cells reacted moderately.

(c) <u>Alkaline phosphatase.</u> Sections of porcine epididymis treated with the alkaline phosphatase technique react

more intensely than with acid phosphatase. The location of the reaction is the same (Figure 55). The surface of the epithelial cells, including the stereocilia, the basement membrane, and the capillaries, react very strongly after 4 hours of incubation. The mass of spermatozoa in the lumen of the tubule react very strongly. The nuclei of the epididymal epithelial cells react slightly. The reaction is similar in all parts of the epididymis studied.

(d) Bauer-Feulgen. Sections of epididymis treated with the Bauer-Feulgen technique for the demonstration of glycogen showed the presence of glycogen granules in the cytoplasm of the epididymal epithelial cells and in the walls of the capillaries associated with the basement membrane. This was observed in all three areas of the epididymis studied. The glycogen reaction was not present in control sections which were incubated in saliva or diastase for one hour at 38 degrees Centigrade. Sections from the head of the epididymis which contained the vacuoles reacted strongly in the vacuole areas (Figures 56 and 57). The greatest amount of glycogen was observed in the tallest epithelial cells in the tail of the epididymis (Figures 59 and 100).

(e) Periodic acid-Schiff. In sections of the epididymis from which the glycogen had been removed by diastase solution at 38 degrees Centigrade, two areas, the surface of the epi-

thelial cells and the basement membrane, reacted to the PAS treatment. The spermatozoa in the lumen of the tubule cross sections showed a reaction in the acrosome.

(f) <u>Oil red O</u>. Sections of epididymis, prepared by freezing microtome technique and treated with the oil red O technique, revealed that the epithelial cells contained numerous small lipid droplets in the distal cytoplasm, giving that half of the cell nearest the lumen an orange color. The secretion droplets associated with the stereocilia and those that were free in the lumen also reacted (Figures 60, 61, 97 and 98).

(g) <u>Sudan black B</u>. No reaction was noted in the epididymal epithelial cells after treatment with sudan black B. The middle piece of the spermatozoa in the lumen of the tubule sections showed some reaction.

(h) <u>Gomori's silver</u>. In sections of epididymis which have been prepared by the Gomori method of silver impregnation, it can be observed that the basement membrane is composed chiefly of reticular fibers, which are continuous with the larger collagenous fibers. Capillary channels can be seen throughout the reticular network under and in intimate relationship with the epithelium.

3. Vas deferens

(a) General histological observations. The vas deferens
is made up of a lumen lined by simple columnar epithelium.
The epithelium appears to be secretory in nature, contributing
droplets of material into the lumen. Under the undulating
epithelium, a thick capillary network is related to the base-
ment membrane. A very thick collagenous muscular coat makes
up the bulk of the vas deferens (Figures 64 and 65). The
lumen varies from 700-900 micra. Near the epididymis, the
epithelium of the vas deferens is highest. The farther re-
moved from the testicle, the lower the epithelium becomes.

(b) Acid phosphatase. After 24-48 hours of incubation
with the acid phosphatase technique, the surface of the
epithelium of the vas deferens reacts. The capillaries mixed
with the basement membrane under the epithelium react to a
lesser extent (Figure 62).

(c) Alkaline phosphatase. The reaction for alkaline
phosphatase is more distinct than for acid phosphatase. After
4 hours of incubation with the alkaline phosphatase technique,
the surface of the epithelium is very reactive. The capil-
laries and blood vessels under the epithelium react to a
lesser degree (Figure 63).

(d) <u>Glycogen</u>. There are a great number of glycogen granules in the walls of the blood vessels under the epithelium and in the epithelial cells lining the vas deferens. This reactive material is not present in sections which have been incubated in saliva for one hour at 38 degrees Centigrade.

(e) <u>PAS</u>. The most PAS positive reaction is on the surface of the epithelial cells. The next strongest reaction is in the capillary walls under the epithelium.

(f) <u>Oil red O</u>. No reaction was observed in sections of vas deferens prepared by freezing, sectioning, and treatment with oil red O technique.

(g) <u>Sudan black B</u>. No reaction in any part of the vas deferens was noted after treatment with sudan black B.

(h) <u>Gomori's silver</u>. A very fine reticular network, associated with the capillary system under the epithelium, can be observed with the Gomori silver technique. The reticular fibers blend with a layer of collagenous fibers, which form a dense layer around the epithelial tube. Reticular fibers mixed with muscle tissue form the outer structure of the vas deferens.

C. Histological Studies of the Female Genital Tract

1. Uterus

(a) General histological observations. The findings of this study were found to conform closely with the description of the sow's uterus during estrus by Corner (1921). The epithelium is characterized by a columnar appearance, although some of the cells do not seem to extend all the way to the lumen (Figures 66 and 67). The thickness of the epithelium is 25-30 micra. Vacuole-like areas are evident in the basal region of the epithelium. Corner describes these areas as a phenomenon of degeneration where two or three cells become vague of outline, with chromatolysis of the nuclei, so that a small vacuole is formed in which lie a few nondescript nuclear fragments or granules (Figure 72). Mitotic figures are regularly seen in the surface epithelium lining the uterus (Figures 68 and 69). Large numbers of neutrophilic poly-morphonuclear leucocytes are evident in the subepithelial connective tissue (Figure 70); some are embedded in the epithelium, making their way toward the lumen. There are some areas where the leucocytes are clumped and extend to the surface (Figure 71). Some sections show leucocytes in the lumen (Figure 70).

The connective tissue stroma under the epithelium is very edematous and loose. There are many capillaries and edematous

spaces near the surface epithelium (Figure 66). The reticular network on which the epithelium rests is perforated by many capillaries (Figure 67).

The superficial glandular tubules are ciliated (Figure 72). Many secretory glandular tubules are embedded in the deeper layers of the stroma (Figures 66 and 74). Vacuolar areas suggestive of degeneration were observed in the glandular epithelium (Figure 75).

(b) Acid phosphatase. The surface of the uterine epithelium is the most reactive (Figure 76). This reaction extends down into the cytoplasm of the cells, one third of the way to the basement membrane. The capillaries beneath the epithelium are reactive. The leucocytes in and under the epithelium react. The luminal border and the basement membrane of the endometrial glandular tubules react also.

(c) Alkaline phosphatase. The surface of the uterine epithelium is very reactive. The capillaries and blood vessels under the epithelium are reactive also (Figure 78). The basement membrane of the glandular tubules and their associated capillaries and blood vessels react distinctly (Figure 79).

(d) Glycogen. There are a few glycogen granules near the surface of the epithelial cells lining the uterus. Also glycogen granules are present in the leucocytes and in the degenerative vacuolar areas of the epithelium (Figure 77).

The glandular tubular epithelial cells contain abundant glycogen in their cytoplasm as well as the walls of the capillaries and blood vessels related to them. There were no glycogen granules in control sections incubated in saliva or diastase for one hour at 38 degrees Centigrade.

(e) PAS. The only areas which show coloration after incubation in diastase and treatment with the PAS technique were the reticular tissue fibers in the basement membrane of the surface and glandular epithelium. Other connective tissue elements in the loose stroma of the uterus show a lesser reaction.

(f) Sudan black B. Both the surface epithelium and glandular epithelium of the endometrium are quite reactive. The endothelium of the capillaries and blood vessels and the erythrocytes are also reactive.

(g) Gomori's silver. The reticular nature of the basement membrane of the surface epithelium, glandular epithelium, and capillary walls is very well demonstrated (Figures 80 and 81).

2. Oviduct

(a) General histological observations. The structure of the oviduct varies from one area to another. The oviduct nearest the uterine horn has a relatively simple lumen which

has 10-12 folds of epithelium and connective tissue stroma extending into it (Figure 84). The outside diameter is 175 micra. In the middle region of the oviduct, the lining epithelium is thrown up into long compound villi type projections into the lumen. The outside diameter of the oviduct is enlarged to 320 micra, almost twice that of the segment nearest the uterus. This suggests the beginning of the ampulla or dialated portion of the oviduct. The third segment is 725-750 micra in diameter and the lumen is complicated by numerous folds of connective tissue stroma covered by epithelium (Figure 82).

The epithelium is similar in all three segments of the oviduct, 20-30 micra in thickness. It is composed of simple columnar ciliated epithelium with large basally located nuclei. Some of the cells show evidence of secretory activity at the luminal surface. Others show no secretory activity and have a ciliated border next to the lumen (Figure 83). The ciliated cells are the most plentiful and are most evident with trichrome staining. Mitotic activity can be observed at the basal region of the epithelium.

The folds extending into the lumen are filled with a loose connective tissue stroma which is well supplied with capillaries and blood vessels.

(b) Acid phosphatase. The first segment of the oviduct nearest the uterine horn shows a reaction after 48 hours in-

cubation with acid phosphatase technique. This reaction appears as granules in the cytoplasm of the epithelial cells near the lumen.

The second and third segments, where the folds become extensive in the formation of the ampulla, react only in the connective tissue stroma of the folds. The capillaries and blood vessels are also very reactive.

(c) <u>Alkaline phosphatase</u>. After 4 hours incubation with the alkaline phosphatase treatment, the first segment or isthmus of the oviduct shows a reaction on the surface of the epithelium and also in the capillaries and blood vessels in the connective tissue stroma under the epithelium (Figure 84).

The second and third segments do not show any reaction in the epithelium. The connective tissue stroma, blood vessels, and capillaries of the folds show a strong reaction (Figure 85).

(d) <u>Glycogen</u>. Using the Bauer-Feulgen technique, all three segments of the oviduct were observed to contain many small glycogen granules in the cytoplasm of the epithelial cells nearest the lumen. Some of the finer granules are present down to the nuclear level and in some instances to the region of the basement membrane (Figures 86, 87, and 99). These granules are not present in sections incubated for one

hour at 38 degrees Centigrade in diastase solution prior to PAS treatment.

(e) <u>PAS</u>. Glycogen granules are apparent with PAS treatment in a location similar to that found with the Bauer-Feulgen technique. In control sections incubated in diastase, the glycogen granules are not present. The only structures that are stained red after diastase treatment are the reticular fibers of the connective tissue stroma under the epithelium.

(f) <u>Sudan black B</u>. The strongest reaction in sections of oviduct treated with sudan black B was in the erythrocytes.

A variable reaction of the surface epithelium was noted. This ranged from a diffuse darkening of the entire cytoplasm and cell boundaries of the epithelial cells to very fine discrete black droplets or granules in the cytoplasm.

(g) <u>Gomori's silver impregnation</u>. The reticular fibers making up the basement membrane can be observed as a dense network under the epithelium. The reticular fibers appear to be continuous with the collagenous fibers of the connective tissue stroma in the irregular folds of the oviduct.

3. <u>Ovary</u>

(a) <u>General histological observations</u>. All of the sows used for histological material were slaughtered within 27 hours after natural copulation. The ovarian material repre-

sents ovaries during the period of estrus. The sections of ovarian tissue showed developing and ripe follicles in the cortex of the ovarian tissue. The cavity of the follicles was lined by granulosa cells or follicular epithelium and this was surrounded by the theca interna and externa. There was a great amount of regressing luteal tissue which probably represented luteinized atretic follicles or remnants of corpora lutea from the previous ovulation. Some of these masses of regressing luteal tissue contained amorphous masses of colloidal material in their center and others contained pigment granules.

(b) Acid phosphatase. A very slight reaction was observed in theca interna cells of developing and mature follicles after 24-48 hours incubation.

(c) Alkaline phosphatase. A very distinct reaction was observed in the theca interna cells around developing and mature follicles. Also the numerous capillaries and blood vessels in this area react. There was very little reaction in the regressing luteal tissue, only an occasional capillary.

(d) Glycogen. Blood vessels around follicles show fine glycogen granules in their walls. Some glycogen granules were present in the germinal epithelium. These were not present in control sections incubated in diastase.

(e) PAS. Connective tissue fibers of the cortical stroma under the germinal epithelium and around the follicles are quite red. Reactive fibers are also distributed throughout the regressing luteal tissue.

(f) Sudan black B. The granulosa cells and the germinal epithelial cells react with about the same intensity as erythrocytes. In some sections there are many droplets in the cytoplasm of what appear to be connective tissue cells outside the theca externa of mature follicles (Figure 88). In most cases these cells are closely related to capillaries or blood vessels. The droplets are more reactive than erythrocytes. There were also some of these fat laden cells in what appeared to be regressing luteal tissue. The cells that contain these droplets are very diffusely distributed with no regularity as to location. They seem to be irregular cells like fibroblasts.

(g) Gomori's silver. Reticular fibers are evident in the basement membrane of the germinal epithelium, blood vessels, and capillaries. A reticular network is also interspersed with the regressing luteal tissue and around follicles with the thecal cells. The area around the follicles and in the luteal tissue contains many capillaries.

D. Fate of Seminal Fluid and Spermatozoa after Insemination

Five sows were used to study the changes that take place in the seminal fluid and spermatozoa after it is deposited in the genital tract by natural breeding. Observations from these sows are organized in Table 2 to show the sequential changes that take place following breeding.

Ten minutes after breeding a complete gelatinous plug was found in the cervix. Thirty five ml. of fluid material was drained from the left uterine horn. Smears made from this material revealed a very high number of spermatozoa. Spermatozoa were observed in the lumen of sections taken from all three locations of the right horn. Sperm were recovered from the middle portion and that part adjacent to the uterine horn of the left oviduct by means of the flushing technique. In histological sections of the right oviduct, spermatozoa were observed only in the middle section.

Thirty minutes after breeding the complete plug was present in the cervix. Ten ml. of fluid material was drained from the left uterine horn. Smears made from this material showed a great number of spermatozoa and a large number of leucocytes. In sections taken from three locations of the right horn, spermatozoa were observed in the lumen of all three areas. Spermatozoa were found in the segment of the left oviduct adjacent to the uterine horn by means of the flushing technique. Spermatozoa were observed in section one

Table 2. Summary of observations of fate of seminal fluid and spermatozoa in the female genital tract after natural breeding

Sow	Time after breeding	Age of sow	Breeding history	Mucous plug	Volume of fluid in left horn	Cells on smears	Areas where spermatozoa were observed		
							Left oviduct flushing 1 2 3 4	Right horn uterine sections 1 2 3	Right oviduct sections 1 2 3
4	10 min.	5 mo.	virgin	present	35 ml.	SSSS[a]	S S - -	S S S	- S -
3	30 min.	16 mo.	1 litter 3 mo. before	present	10 ml.	SSSL[b]	S - - -	S S S	S S -
1	105 min.	16 mo.	1 litter 3 mo. before	came out before slaughter	only foam	SSLL	S S - -	- S -	S S -
5	8½ hours	16 mo.	1 litter 3 mo. before	none	none	SLLL	S - - -	- S S	- - -
2	27 hours	5 mo.	virgin	none	none	LLLL	- - - -	- - -	- - -

[a] S - sperm.

[b] L - leucocytes.

and section two of the histological sections of the right oviduct. No spermatozoa were found in sections of the ampulla.

One hour and 45 minutes after breeding, no mucous plug was found in the cervix. The plug was found in the stable where the sow was kept before she was slaughtered. When the left uterine horn was opened, no fluid could be removed. Only a slight amount of foamy moisture was present in the lumen. Smears of this material revealed a fewer number of spermatozoa than the previous sows studied and a greater number of leucocytes. Many leucocytes were phagocytizing or had phagocytized spermatozoa (Figure 90, 91, and 92). Spermatozoa were observed in the lumen of histological sections prepared from the middle section of the right uterine horn. Spermatozoa were found upon flushing the first and second segments of the left oviduct. In histological sections of the right oviduct, spermatozoa were observed in the first and second segments.

Eight and one half hours after breeding, no mucous plug was found in the cervix. No evidence of the plug was found in the stable where the sow was kept until she was slaughtered. When the left horn was opened to remove uterine contents, there was only a slight amount of moisture. Smears were made by drawing a glass slide across the exposed surface of the endometrium mucous membrane. There were more leucocytes observed on these preparations than spermatozoa. In histological sections of the right uterine horn, spermatozoa were observed

in the lumen of the second and third sections. No spermatozoa were observed in the lumen of any of the microscopic sections of the right oviduct. The flushing technique used on the left oviduct revealed just a few spermatozoa from the first segment of the oviduct.

Twenty seven hours after breeding, no mucous plug was present in the cervix. There was no fluid in the left horn. Smears made from the endometrium contained an abundance of leucocytes and only a few spermatozoa, two or three on an entire slide. In histological sections of the right horn, no complete sperm were observed in any of the areas studied. There were a few sperm-cell tails observed but no complete spermatozoa. In histological sections of the oviduct, no spermatozoa were found. There were some fragments that resembled tails in the middle segment of the oviduct. Examination of saline solution flushed through the segments of the left oviduct revealed no spermatozoa.

E. Physiological Observations of Boar Semen

The observations made from the semen collected from the two boars during the summer of 1956 are summarized on Table 3. Boar number one was one year old when collections were started and boar number two was one and one half years old. The younger boar routinely produced a smaller volume of semen than did the older boar. The younger boar averaged 243 ml.

Table 3. Summary of physiological data collected from semen samples from 2 boars on a collection schedule of twice a week

Collection	Boar number	Date	Volume before filtering	Volume after filtering	Weight of gel mass	pH	Specific gravity	Motility	Sperm-cell count /mm.^{3}	Color
1	2	6-20-56	380	307	67	7.4	1.010	4	100,000	White
2	1	6-22-56	213	183	58	7.5	1.010	5	94,700	White
3	2	6-22-56	430	370	98	7.5	1.005	5	178,000	White
4	1	6-25-56	205	188	56	7.4	1.015	5	170,000	White
5	2	6-25-56	440	372	100	7.5	1.011	4	121,600	Red
6	1	6-29-56	210	189	52	7.6	1.010	4	114,100	White
7	2	6-29-56	390	368	83	7.4	1.011	5	122,100	Pinkish
8	1	7- 2-56	232	190	58	7.3	1.015	5	177,500	White
9	2	7- 2-56	460	370	101	7.3	1.015	5	125,000	Pink
10	1	7- 6-56	228	153	53	7.2	1.010	5	123,700	White
11	2	7- 6-56	395	360	66	7.2	1.010	5	79,700	Off white (yellow)
12	1	7- 9-56	265	215	54	7.3	1.015	5	88,350	White
13	2	7- 9-56	400	330	70	7.3	1.010	5	103,400	Pinkish white
14	1	7-13-56	250	190	54	7.2	1.010	5	156,900	White
15	2	7-13-56	452	414	86	7.2	1.010	5	114,600	Off white
16	1	7-16-56	277	230	62	7.3	1.010	5	201,600	White
17	2	7-16-56	555	432	127	7.3	1.008	5	81,200	White
18	1	7-20-56	275	230	62	7.25	1.010	5	135,800	White
19	2	7-20-56	645	535	113	7.35	1.010	5	86,800	White
20	1	7-23-56	207	150	67	7.2	1.015	5	97,900	White
21	2	7-23-56	477	390	104	7.2	1.010	5	167,500	White
22	1	7-27-56	310	240	70	7.1	1.015	5	152,300	White

Table 3.   (Continued)

Col- lec- tion	Boar num- ber	Date	Volume before filter- ing	Volume after filter- ing	Weight of gel mass	pH	Specific gravity	Motil- ity	Sperm-cell count /mm.^{3}	Color
23	2	7-27-56	435	340	99	7.1	1.008	5	133,500	White
24	1	7-30-56	205	160	49	7.3	1.010	5	252,200	White
25	2	7-30-56	325	228	102	7.4	1.010	5	262,800	White
26	1	8- 3-56	283	208	79	7.3	1.015	5	123,600	White

and the older boar averaged 445 ml. The average weight of the gel mass for the younger boar was 59.5 gms. and for the older boar 93.5 gms.

The highest specific gravity of semen was the same for both boars - 1.015. The lowest for the younger boar was 1.010 and the lowest for the older boar was 1.005. The average specific gravity for the younger boar was 1.012 and for the older boar - 1.009.

The highest hydrogen ion concentration in the semen of the youngest boar was pH 7.6 and in the older boar it was 7.5. The lowest was pH 7.1 in both boars. The average pH of the semen was 7.38 for the younger boar and 7.32 for the older boar.

For the most part the color of the semen was milky white. Boar number two, the larger and older boar, produced very red semen June 25, 1956. Erythrocytes were observed on microscopic examination of the semen. This red discoloration continued to a lesser degree for the next 5 collections, only showing a yellowish to off-white discoloration. Erythrocytes could still be demonstrated microscopically.

All samples collected were completely odorless.

The highest sperm-cell count was found in the oldest boar, 262,800 per mm.[3]. The highest count found in the young boar was 252,200 per mm.[3]. The lowest sperm-cell count found in the young boar was 88,350 per mm.[3]. The lowest in the

older boar was 79,700 per mm.^{3}. The average count for the older boar was 129,000 spermatozoa per mm.^{3}. The average sperm-cell count in the younger boar was 145,280 per mm.^{3}.

Spermatozoa, which had protoplasmic droplets connected either proximally or distally to the middle piece, were observed in all samples of semen studied. Some spermatozoa were observed which had a damaged or distorted galea capitis. Very few bent or curled tails were observed in fresh semen. After prolonged storage, these abnormal forms did appear. Boar semen has a great number of crystalline particles suspended in the fluid portion. Upon standing, these crystals settle out together with the spermatozoa to form a layer on the bottom of the container.

The motility of the spermatozoa in the various semen samples was determined subjectively by microscopic observation. The best samples were rated 5. If no spermatozoa are motile the rating is one. All but three of the samples studied were rated 5; nearly all sperm with a high degree of progressive motion. These three were rated 4; most with progressive motion. The motility was very vigorous.

The live-dead staining technique of Lasley, Easley, and McKenzie (1944) was used on some of the semen samples. The results obtained gave variable results. Samples of fresh semen would have 60-70 per cent of the spermatozoa stained, presumably evidence that the sperm were dead. The same

sample after storage for 24 hours had only 30-40 per cent of the sperm stained. The results of this technique were so unpredictable that this method of evaluation was discontinued.

Before the boars were castrated for histological studies of the genital tracts, a series of semen samples was studied to compare the ejaculated semen with epididymal fluid. The results of these preliminary studies are presented in Table 4.

Table 4. Summary of pH and sperm cell counts of ejaculated semen

Boar number	Average sperm density	Average pH
Boar # 2	290,286 per mm.^{3}	7.32
Boar # 3	188,576 per mm.^{3}	7.34
Boar # 5	210,400 per mm.^{3}	7.30
Boar # 6	271,720 per mm.^{3}	7.35

When epididymal fluid was collected and studied from these same boars, the data shown in Table 5 were collected.

F. Artificial Breeding Experiments

During January and February 1957, 4 gilts were artificially inseminated on the second day of heat with 100 ml. of fresh semen within 15 minutes after collection. They all became pregnant and had litters of variable size; 13, 5, 17, and 3. In this preliminary trial, the conception rate was

Table 5. Summary of pH and sperm cell counts of epididymal fluid

Boar number	Average sperm density	Average pH
Boar # 2	5,140,000 per mm.^{3}	6.65
Boar # 3	4,820,000 per mm.^{3}	6.45
Boar # 5	5,340,000 per mm.^{3}	6.45
Boar # 6	6,580,000 per mm.^{3}	7.05

100 per cent and the size of the litters was within the range found in natural breeding.

During December 1957 and the first week in January 1958, 40 female swine were inseminated artificially at the Napier Swine Breeding Farm. The boars from which the semen was collected for this breeding were brought to the Obstetrics Laboratory of the Veterinary Division, Iowa State College, on November 21, 1957. Two Yorkshire boars, one Duroc, and one Poland China were used. By December 3, 1957, three of the boars were serving the dummy sow satisfactorily. December 5, 1957, the first sows were inseminated at the Swine Breeding Farm, 7 miles from the Obstetrics laboratory. Tables 6 and 7 give the breeding data on the 40 sows inseminated.

Sixteen females or 40 per cent of the animals inseminated conceived after the first service (Table 6). Nine sows or 23 per cent conceived after the second service. A total of 25 sows or 63 per cent inseminated artificially became preg-

Table 6. Summary of artificial breeding information on sows which conceived

Sow number	Breed	Age months	First[a] breeding date	Min. after collection	Vol.	Quality	Second breeding date	Min. after collection	Vol.	Quality	Farrowing[b] date	Number pigs born	3 week litter
1157	Y	8	12-21	75	75	G	-------	--	---	-	4-12	10	8
560	D	8	12-21	60	150	G	-------	--	---	-	4-15	8	7
30	D	8	12-22	35	55	G	-------	--	---	-	4-14	11	10
40	P	20	12- 6	115	100	G	-------	--	---	-	3-30	4	4
36	P	32	12- 6	125	100	G	-------	--	---	-	4- 3	10	8
1201	Y	8	12- 6	90	75	G	-------	--	---	-	3-15	8	0
1447	P	8	12- 7	55	75	P	-------	--	---	-	4- 2	9	8
2297	D	14	12-10	70	100	G	-------	--	---	-	4- 4	6	4
1446	P	8	12-17	70	200	M	-------	--	---	-	4-13	5	5
1717	Y	14	12-19	60	120	G	-------	--	---	-	4-12	15	13
564	D	8	12-19	30	120	G	-------	--	---	-	4-14	8	8
243	P	8	12-19	60	60	M	-------	--	---	-	4-15	11	7
775	P	8	12-19	65	65	F	-------	--	---	-	4-13	10	4
198	Y	8	12-19	80	100	G	-------	--	---	-	4-12	11	7
2212	Y	14	12-21	70	75	G	-------	--	---	-	----[c]	--	-
105	P	20	12-16	50	100	M	-------	--	---	-	----[d]	--	--

[a]All dates were 1957.

[b]All dates were 1958.

[c]Died 2-23-58 pregnant.

[d]Died 3-23-58 pregnant.

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Table 6. (Continued)

Sow number	Breed	Age months	First breeding date	Min. after collection	Vol.	Quality	Second breeding date	Min. after collection	Vol.	Quality	Farrowing date	Number pigs born	3 week litter
196	Y	8	12-18	50	200	G	1- 6-58	55	130	G	e		
908	P	8	12-18	40	100	M	1- 2-58	58	135	G	4-29	3	3
17	Y	8	12-17	85	100	G	1- 6-58	50	130	G	4-30	5	3
2607	Y	14	12- 6	120	75	G	1- 2-58	48	140	G	4-27	16	9
2211	Y	14	12- 5	95	100	G	1- 3-58	30	260	G	4-30	17	8
1738	Y	14	12- 5	85	100	G	12-26-57	45	115	G	4-18	13	11
904	P	8	12- 7	50	75	P	12-27-57	40	180	P	4-19	9	4
4298	D	8	12-10	60	100	G	12-31-57	30	100	G	4-25	8	3
1497	Y	8	12-10	55	140	G	12-30-57	20	120	G	4-23	12	5

eDied 3-9-58 pregnant.

Table 7. Summary of artificial breeding information on sows which did not conceive

Sow number	Breed	Age	First breeding	Min. after collection	Vol.	Quality	Second breeding	Min. after collection	Vol.	Quality	Returned in heat not bred	Remarks
235	P	8	12-16-57	60	100	M	--------	--	---	-	1-28-58	no litter
233	P	8	12- 5-57	60	170	G	--------	--	---	-	1-13-58	no litter
526	D	8	12- 7-57	50	95	G	--------	--	---	-	1-13-58	no litter
225	P	20	12-18-57	50	100	M	--------	--	---	-	-------	no litter
236	P	8	12-22-57	60	125	P	--------	--	---	-	1-10-58	no litter
158	D	8	12-16-57	50	70	G	1- 2-58	40	115	G	1-28-58	no litter
1711	Y	14	12- 7-57	45	100	G	12-30-57	30	200	G	1-19-58	no litter
2425	Y	14	12- 7-57	40	100	G	12-26-57	55	50	G	1-17-58	no litter
2301	D	8	12- 7-57	45	95	G	12-26-57	50	110	G	1-15-58	no litter
1767	Y	14	12- 6-57	105	75	G	12-26-57	50	115	G	1-15-58	no litter
404	Y	8	12- 6-57	75	75	G	12-26-57	50	50	G	1-13-58	no litter
179	Y	8	12- 5-57	105	100	G	12-26-57	55	115	G	1-13-58	no litter
1200	Y	8	12-17-57	75	100	G	1- 6-58	55	230	G	1-26-58	no litter
1299	Y	8	12-17-57	65	100	G	1- 6-58	60	130	G	1-25-58	no litter
77	D	8	12-16-57	45	70	G	12-22-57	40	55	G	1-27-58	no litter

nant. Figures 93, 94, and 95 show sows which were inseminated artificially with their litters. Seventy five per cent of the sows bred naturally for spring litters at the Napier farm became pregnant.

V. DISCUSSION

A. Life History of Spermatozoa

The significance of the presence of acid and alkaline phosphatase in the Sertoli cells, in the epididymis and in ejaculated semen is a matter of conjecture. It would seem from the observations made in this work that these enzymes are not incorporated inside the sperm-cell itself, but rather are outside the boundaries of the cell. Possibly the enzymes are bound to the surface of the cell by adsorption where the enzymatic activity takes place; or perhaps, the enzyme is very superficially located in the substance of the sperm-cell. The reaction of spermatozoa, which had been repeatedly washed and centrifuged, was very weak or negative. The enzyme was either washed from the surface or leached from the substance of the sperm-cell. Areas of dried seminal plasma on slides prepared from whole semen are definitely reactive to the phosphatase technique. It would seem that there is a generous quantity of acid and alkaline phosphatase in the seminal fluid.

Phosphatase is a general classification of enzymes and its activity does not necessarily involve any specific sugar or carbohydrate. The function of catalysing the hydrolysis of hexose phosphate to phosphoric acid and the free sugar, as occurs in the transfer of sugars such as glucose 6-phosphate

from the epithelial cells of the intestine to the blood glucose in the capillaries of the intestinal villi, was shown by Dempsey and Deane (1946). Possibly a similar situation exists with the developing spermatid and mature sperm-cell where hexose phosphate is hydrolysed by phosphatase in the metabolism of the sperm-cell or spermatid.

On smears treated with the phosphatase technique, the location of the reaction in the sperm-cell varies. With the acid phosphatase technique, some spermatozoa react most distinctly in the acrosomal area; others in the postnuclear cap region. With alkaline phosphatase, the acrosomal area of the spermatozoa is definitely most reactive; particularly in freshly ejaculated semen. The fact that clumps of spermatozoa near an aggregation of dried seminal plasma reacted most distinctly would support the hypothesis that the seminal plasma contained the enzyme rather than the sperm-cell itself.

The reaction observed with sudan black B technique when used on mature spermatozoa indicates that the middle piece of the sperm-cell contains considerable phospholipid or lipids other than triglycerides. These results are similar to the findings of Melampy, Cavazos, and Porter (1952) in the bovine.

The posterior head cap of the spermatozoa studied showed a greater affinity for silver impregnation than any other part.

The development of the acrosome can be followed in the boar testicle after PAS treatment. These findings were similar to those of Leblond and Clermont (1952b) in the rat and Cavazos (1954) in the bull and ram. The reaction obtained in mature spermatozoa was also similar to that reported in the bull by Cavazos. Since the control smears, which were incubated in diastase for one hour at 38 degrees Centigrade prior to PAS treatment, were no different than those not treated with diastase; it is likely that the substances detected are mucoproteins and mucopolysaccharides.

The Feulgen technique produced a uniformly positive reaction throughout the head and there was no line of demarcation between the acrosome and the postnuclear cap. This is similar to the findings reported by Melampy et al. (1952) in the bull.

After methyl green and pyronine staining, the residual bodies stained a deep red. After treatment with ribonuclease these bodies were not evident. This would lead to the conclusion that the residual bodies observed in the lumen of seminiferous tubules containing late spermatids represent ribonucleic acid being extruded with the cytoplasm of the spermatid. The mature spermatozoa contain no detectable RNA. This is consistent with the thought that the endoplasmic reticulum and microsomes of the spermatid are discarded and make no contribution in fertilization. Most descriptions of

spermiogenesis fail to explain what becomes of the microsomes and endoplasmic reticulum of the early spermatid. There is no structure in the definitive sperm-cell which can be traced back to these cytoplasmic organoids. Pelc (1957) gives auto-radiographic evidence that DNA is synthesized from RNA in mouse testis.

The separation of and wrinkling of the acrosome or galea capitis, observed in spermatozoa that had been stored for a period of time, indicate that degenerative processes may occur due to enzyme or bacterial action. The observations by light microscope methods tend to confirm the electron microscopic observations of Hancock (1956). After 24 hours storage, a great number of motile bacteria could be observed in the seminal fluid. What effect the metabolic by-products of microorganisms have on spermatozoa metabolism is an important problem which should be studied.

With all of the staining techniques, it was observed that the attachment of the tail to the sperm-cell head was off-center in the majority of spermatozoa. This is consistent with the findings of Hancock (1956).

No glycogen was observed in any sperm-cells by either the PAS technique or the Bauer-Feulgen technique. This is similar to the results reported by Melampy et al. (1952) in the bull.

The use of phosphatase studies on semen smears would seem to have limited value in obtaining information as to spermatozoa condition and integrity after storage. There is a great amount of variation in the reaction obtained. This seems to be dependent on the amount of seminal plasma included on the smear rather than the metabolic condition of the sperm-cell.

It is difficult to interpret the results of techniques like silver impregnation and the protargol technique. Why certain parts of the sperm-cell are impregnated more than others is not known. It is likely that, as suggested by Melampy et al. (1952), the coverings of the different parts of the sperm-cell possess dissimilar molecular constitutions and, therefore, may exhibit different adsorptive capacities for various seminal constituents such as enzymes and sub-strates.

B. Male Genital Tract

No observations were made of histological structure of the testicle which have not previously been recorded in the literature.

On sections of testicle prepared with the PAS technique, it was possible to observe that different tubule cross-sections represented different stages of the cycle of the seminiferous epithelium. After careful observations, it was

concluded that the stages of the cycle in the boar testicle correspond quite closely with the stages of the cycle described in the rat by Leblond and Clermont (1952b).

The presence of acid and alkaline phosphatase and glycogen in the cytoplasm of the Sertoli cells in the basement membrane and related capillary walls of the seminiferous tubules was interpreted as evidence that hexose sugars were crossing the cell boundaries at these points as nutrition for the development and maturation of the spermatids. Elftman (1950) emphasized the importance of the mouse Sertoli cell in transferring material from the vascular supply to the lumen of the seminiferous tubules.

The presence of lipid droplets in the basal part of the seminiferous tubules was taken as further evidence of nutritive elements passing through the Sertoli cells to the region of maturing spermatids. Lynch and Scott (1951) relate lipid material in the Sertoli cells to a nutritive function.

The observation of reactions to alkaline phosphatase, phospholipid, and lipid material in the interstitial cells of Leydig was interpreted as evidence of the steroidal hormone precursors. Cavazos (1954) reported large lipid droplets in both the interstitial cells and Sertoli cells of the bull testis.

Many of the capillaries and blood vessels closely associated with the Leydig cells were heavily laden with glycogen

granules. Some of the Leydig cells themselves contained glycogen granules in their cytoplasm. With routine techniques, the periphery of the Leydig cells were mottled and honey-combed with unstained empty vacuoles where the lipids had been dissolved out by xylol.

The Gomori silver impregnation technique demonstrated that the basement membrane of the seminiferous tubule is composed of many fine reticular fibers. Many capillaries are related to this, suggesting the functional role of supplying nutritional elements for spermatogenesis. One could easily understand how an increase in the connective tissue basement membrane of the seminiferous tubules, at the expense of the capillary elements, would interfere with nutrition and normal spermatogenesis.

The presence of acid and alkaline phosphatase, glycogen, and lipid materials in the cytoplasm of the epididymal epithelial cells was interpreted as evidence of secretory activity. Secretion is very evident histologically; as enlargement and filling of the stereocilia and finally expulsion of secretion droplets. Undoubtedly, the secretions of the epididymis are very important for sperm-cell metabolism during the period of storage and maturation in this tubular structure. The pH of epididymal fluid was much lower than that of ejaculated semen. Whether this low pH is due to the epididymal secretions or testicular fluids is not known.

The presence of phosphatase activity and glycogen in the epithelium of the vas deferens was interpreted as evidence of nutrition for the maintenance of the epithelium. The absence of lipid droplets suggests that if secretion occurs in the vas deferens, it is of minor importance and does not include lipid secretions. The primary function of the vas deferens is muscular contraction at ejaculation. The heavy muscular tunic is important for this role.

C. Female Genital Tract

No histological observations were made which are not already well described in the literature.

The presence of acid and alkaline phosphatase areas in the distal cytoplasm and of the surface epithelium in the capillaries beneath the epithelium suggests metabolic activity. A constant source of metabolic fuel is necessary for mitosis and for secretion of the surface epithelium. The glycogen granules in the uterine epithelial cells supports this interpretation. The significance of the reaction for phosphatase and glycogen in the leucocytes invading the epithelium is probably that they are cells with a high rate of metabolism.

The epithelium may be absorptive as well as secretory.

The reaction of the glandular epithelium with phosphatase shows a relationship similar to the surface epithelium, sug-

gesting secretory activity. The significance of the differ-
ence in reaction to phosphatase technique between the isthmus
and ampulla of the oviduct is not understood. In the isthmus,
the reaction is evident in the surface epithelial cells as
well as the underlying capillaries and blood vessels. In the
ampulla, only the vascular channels and connective tissue
under the epithelium is reactive.

All three segments of the oviduct revealed the presence
of glycogen. This indicates that the epithelium of the ovi-
duct has secretory activity. This secretion may be important
to furnish nutrition for the spermatozoa prior to fertiliza-
tion and to the ovum after fertilization. Some very fine
phospholipid droplets were observed in the cytoplasm of the
oviduct epithelial cells.

At the time of heat when this material was collected,
the ovaries are covered by mature follicles. Microscopically
degenerating luteal tissue from the previous heat period were
evident. There were also many small developing and atretic
follicles in the cortical stroma.

Acid and alkaline phosphatase activity was observed in
the theca interna cells. Glycogen granules were present in
the blood vessels around the follicle. This pattern tends to
show the transfer of products for metabolism and secretion of
the internal theca cells. The sudan black B preparations
showed droplets of phospholipid material in what appeared to

connective tissue cells in the theca externa and regressing
luteal tissue. These droplets may indicate the presence of
precursors of steroidal hormones.

D. Seminal Fluid and Spermatozoa after Insemination

The rapid disappearance of the seminal fluid from the
uterine cavity suggests that absorption of the fluid by the
endometrium occurs at a rapid rate. This phenomenon was re-
ported by du Mesnil du Buisson and Dauzier (1955b).

The rapid disappearance of spermatozoa can be attributed
to phagocytosis by the numerous polymorphonuclear leucocytes
present in the lumen of the uterus at estrus. This has been
described by Austin (1957) in rodents and by Joël (1942) in
human beings. Spermatozoa were observed in the neck of some
of the glandular tubules. This would suggest that some of
the spermatozoa may wander off into the blind tubules of the
uterine glands. Mann et al. (1956) suggest that digestion or
enzymic action may destroy the spermatozoa and explain their
disappearance. Observations of free tails in the uterine
lumen 8 to 27 hours after breeding may lend some support to
this theory.

E. Comparison of Ejaculated and Epididymal Fluid

In comparing the sperm-cell concentration and pH of
ejaculated semen and epididymal fluid, it is seen that the

sperm-cell concentration of epididymal fluid is nearly 20 times greater than the sperm-cell concentration of ejaculated semen. The pH of epididymal fluid is lower than the pH of ejaculated semen. The effect this lower pH in the epididymis has on sperm-cell metabolism and motility is not known. Further research should be done to determine the action of the accessory fluid on epididymal spermatozoa. Lasley and Bogart (1944) found that epididymal spermatozoa survived storage and cold shock better than ejaculated spermatozoa.

The results of trials with the live-dead staining technique showed that no regular pattern could be obtained. It was anticipated that the longer time the semen was stored, the higher the percentage of dead spermatozoa would be. This did not follow. A similar finding was reported by Mayer and Oloufa (1951) that none of the live-dead staining methods they used gave consistent results with boar semen.

F. Artificial Insemination

The results obtained in the preliminary trials in artificial insemination were very encouraging. Four sows were inseminated and they conceived and had litters. Later when more extensive breeding trials were conducted under field conditions, the results were not so gratifying. Only 63 per cent of the sows conceived that were inseminated artificially,

as compared to a conception rate of 75 per cent in the herd that was bred naturally.

A recent report by Gotnik et al. (1957) summarizes the results of 6,000 pigs inseminated in Norway and Denmark. The conception rate following inseminations carried out under normal practical conditions was 56 per cent, compared with over 70 per cent for natural service. The authors considered the main problems still to be solved are the preservation of boar semen and the development of an easy method of determining when sows are in heat.

These figures compare favorably with the figures of this research involving smaller numbers. It was felt in this research, that the problem of breeding the sows during the proper time in the estrous cycle was not too great. The sows were inseminated during the second day of heat. The same methods for detecting heat were used for both artificial breeding and natural pen breeding.

The time between collection of the semen and insemination was carefully recorded in each case. The longest period of time, where conception occurred, was two hours and 5 minutes. The smallest volume used was 55 ml. of fresh whole semen. The problem of semen storage is of vital importance in the development of artificial insemination. Preliminary reports on the storage of boar semen in glycine egg yolk buffer are very encouraging; Roy (1955) and Polge (1956).

One problem which was thought to affect the conception percentage was temperature shock. During December, when the insemination was being done, outside temperatures were sometimes below zero. There was no heat in the shed where the actual insemination was done. The glassware became chilled during the insemination procedures. Later in the breeding trials, precautions were taken to keep the semen from becoming chilled by warming the glassware in warm water prior to filling. It was felt that this would eliminate the possibility of temperature shock.

VI. SUMMARY

1. The life cycle of porcine spermatozoa was studied from spermatogenesis in the testicle to the disappearance of the spermatozoa in the uterus.

2. Various histological and histochemical methods were used, including the phase microscope.

3. Five sows were bred naturally and slaughtered after varying intervals of time. Studies of this material included: smears of uterine contents; histological sections of uterus, oviduct, and ovary; and flushing techniques for the detection of spermatozoa in the oviduct.

4. Five boars were castrated to obtain material to study smears of epididymal fluid and histological sections of testicle, epididymis, and vas deferens.

5. A total of 25 ejaculations were studied from two boars in order to collect physiological data on normal boar semen.

6. A total of 44 sows were artificially inseminated in order to learn more about the field problems involved in artificial insemination of swine.

7. The histochemical reactions of the male and female genital tract are described and illustrated.

8. The histological and histochemical reactions of ejaculated spermatozoa are described and illustrated.

9. The possible physiological role of the various parts of the male and female genital tract in furnishing nutrition for sperm-cells is discussed.

10. A comparison is made of epididymal fluid and ejaculated semen and some of their differences are discussed.

11. The results of artificial breeding trials are summarized and discussed.

12. The equipment and methods developed for collecting semen and insemination of sows are described and illustrated.

13. The development of the head cap or acrosome of the porcine spermatozoon as shown by PAS technique is described and illustrated.

VII. CONCLUSIONS

1. There is a great amount of variation in the reaction of porcine spermatozoa for alkaline and acid phosphatase. This is related to the amount of seminal plasma adhering to the sperm cells. Sperm cells washed by repeated suspension in saline and centrifuging showed little or no reaction.

2. After 24-48 hours of storage, the galea capitis became detached or damaged in many spermatozoa. The attachment of the middle piece to the head is off-center in a great number of boar spermatozoa.

3. No glycogen was demonstrated in the sperm-cell at any stage of its life history. Sudanophilic phospholipids were present in the middle piece and protoplasmic droplet.

4. A high percentage of ejaculated spermatozoa possess a protoplasmic droplet either in a distal or proximal position.

5. The aceto-carmine technique for fixing and staining sperm-cells was found to be the easiest to use and the most useful for showing the fine details of sperm-cell morphology.

6. Spermiogenesis and differentiation of the head cap can easily be followed in sections of boar testicle after using the periodic acid-Schiff technique. The acrosomal area and protoplasmic droplet of the definitive sperm-cell are reactive to PAS.

7. The presence of acid and alkaline phosphatase reaction and glycogen in the cytoplasm of the Sertoli cells and in the basement membrane of the seminiferous tubules was interpreted as evidence of the metabolic pathway of materials for spermiogenesis. The presence of lipid droplets in the basal cytoplasm of Sertoli cells was considered to support this interpretation.

8. Many fine capillaries are closely related to the basement membrane of the seminiferous tubules. The functional role of these capillaries is believed to be supplying nutrition for spermatogenesis.

9. Secretory activity of the epididymal cells was demonstrated by an abundance of lipid, glycogen, and phosphatase in the cytoplasm. Further evidence of secretion is the swelling and detachment of the extremities of the stereocilia to form droplets in the lumen. Undoubtedly, the secretions of the epididymis are very important for sperm-cell metabolism during storage and maturation.

10. The presence of acid and alkaline phosphatase and glycogen in the surface epithelium of the uterus, glandular tubules, and oviduct is evidence of the secretion function. Secretions at the time of estrus must have an important nutritive role for spermatozoa. The bulk of the fluid portion of the semen is absorbed within 8 hours after service. Where this fluid goes and what its effect might be on the uterus

or female endocrine balance is not known. If nutrition for the spermatozoa is not provided by the seminal fluid, perhaps it is supplied by secretions of the uterus and oviduct.

11. The major cause of the rapid disappearance of spermatozoa from the genital tract of the sow was found to be phagocytosis by polymorphonuclear leucocytes, which are abundantly present in the lumen of the uterus at the time of heat. Other methods of disappearance of spermatozoa may be by entering glandular tubules and enzyme digestion. Eight to 27 hours after breeding, sperm-cell tail fragments were found in the lumen of the uterus. Very few intact sperm were present 27 hours after service.

12. No spermatozoa were observed penetrating the uterine epithelium.

13. Preliminary trials with artificial insemination, using 4 sows, produced 100 per cent conception at the first service. More extensive trials involving 40 sows resulted in 63 per cent conception. Twenty three per cent of these required two services for conception.

14. More research should be done on epididymal fluid and ejaculated semen to determine what substances are best suited as an extendor and nutrient buffer for spermatozoa preservation.

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IX. ACKNOWLEDGMENTS

X.   ILLUSTRATIONS

Figure 1. Plans for construction of dummy sow

        a. Side view
        b. Hind view of section B
        c. Method of construction of sections

Hooks - 1" apart

9" 12"

←3"→

1½"

B.

←—13"—→

Hooks
1"apart

14½"

5½"

C.

←2½→ ←——13"——→

C.

¾"

B.

Type of
hook used.

Hooks

16½"

5"

1½"

16"

5½"

1½"

9"

⅜" holes - 1" apart

2"

9½"

A.

½"   ¾"

←3"→←3"→1⌐ ⌐1←3"→1⌐   ←————36"————→

←————————————84"————————————→
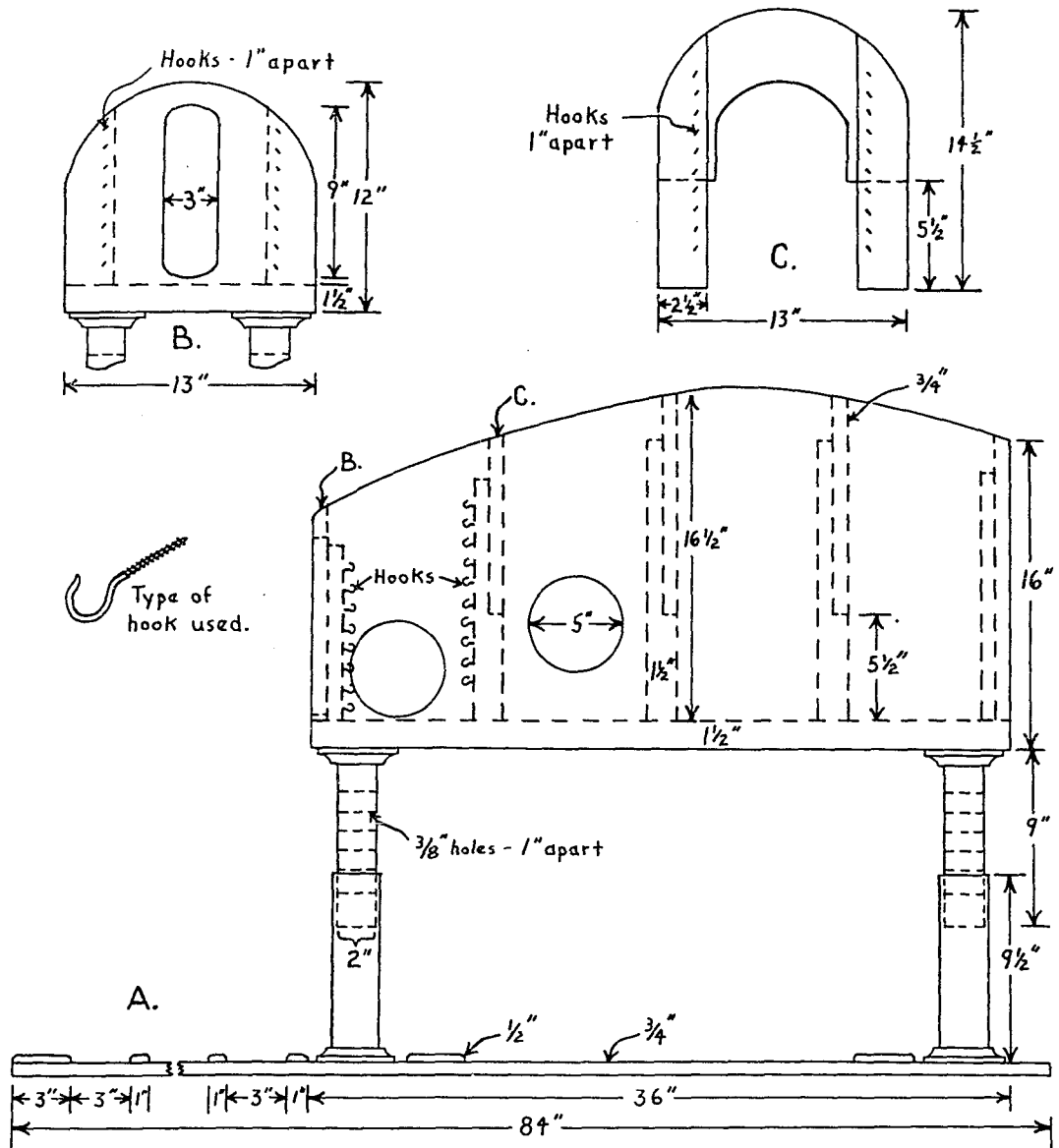
Figure 2. Dummy sow completed with artificial vagina
suspended inside

Figure 3. Hind view of dummy sow and artificial vagina
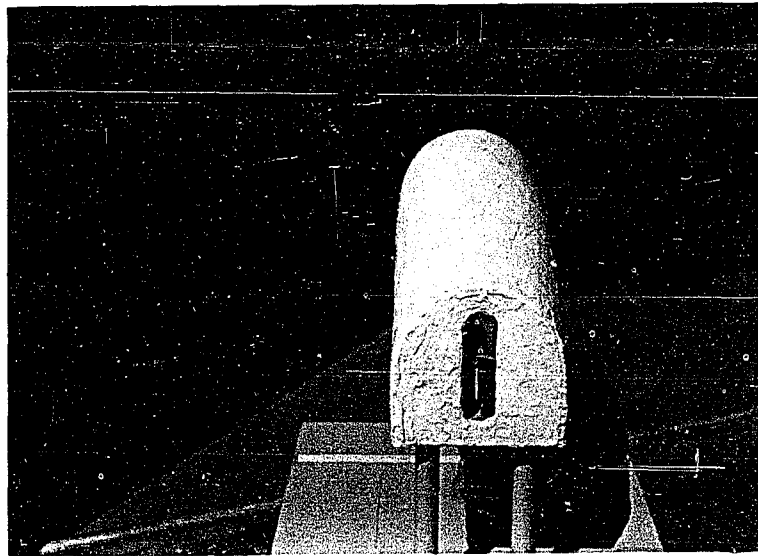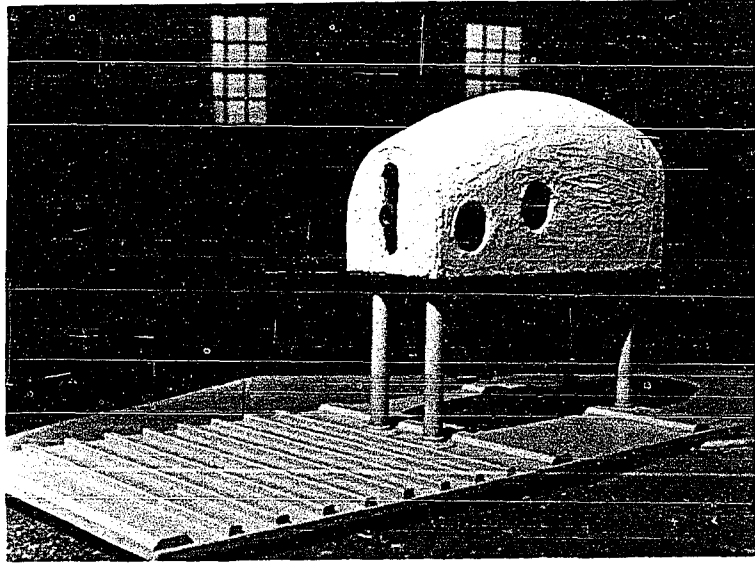
Figure 4.   Component parts of artificial vagina

       1.   Collecting vial
       2.   Sterile gauze
       3.   Gooch tubing connecting cuff, 8 inches long
           and two inches wide
       4.   Eight inch length of one inch radiator hose
       5.   Ten inch piece of one inch gooch tubing
       6.   Rubber bands
       7.   Short piece of gooch tubing to cover hole
           in radiator hose

Figure 5.   Artificial vagina assembled

       1.   Inner liner is inside radiator hose and
           ends reflected
       2.   Short piece of gooch tubing placed over hole
           in radiator hose
       3.   Gauze is placed over mouth of vial
       4.   Connecting cuff is placed over vial and
           end of vagina
       5.   When this assembly is used the boars penis
           extends into the flexible cuff where
           pressure may be applied by the operators
           hand
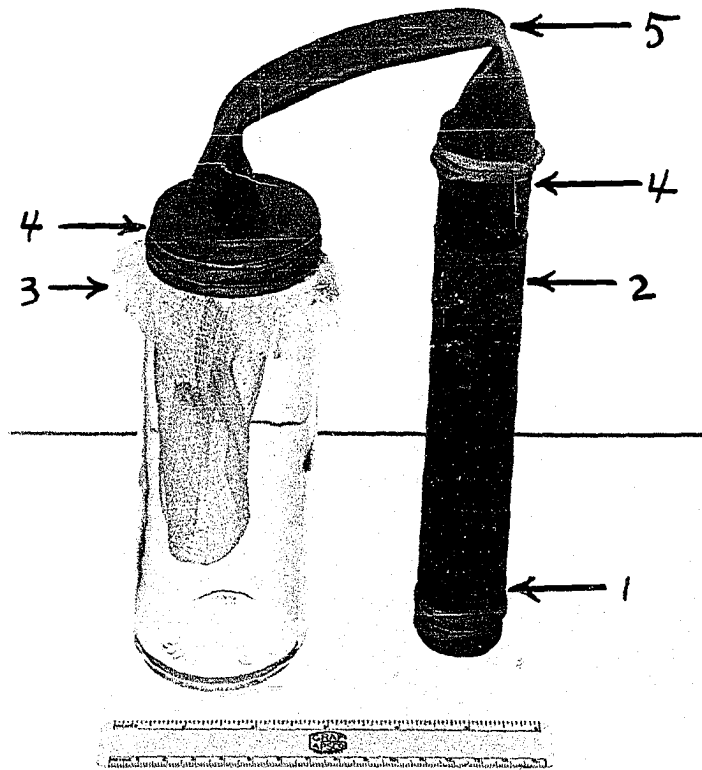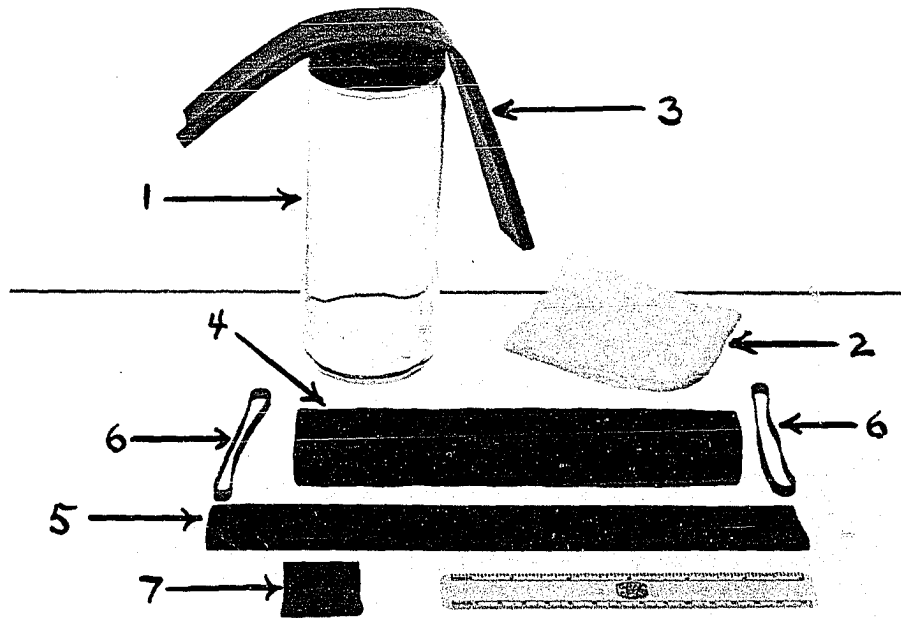
Figure 6. Artificial sow with artificial vagina fixed
            inside - rubber tubing is collapsed and empty

Figure 7. Artificial sow being used - semen is accumulating
            in the rubber tubing
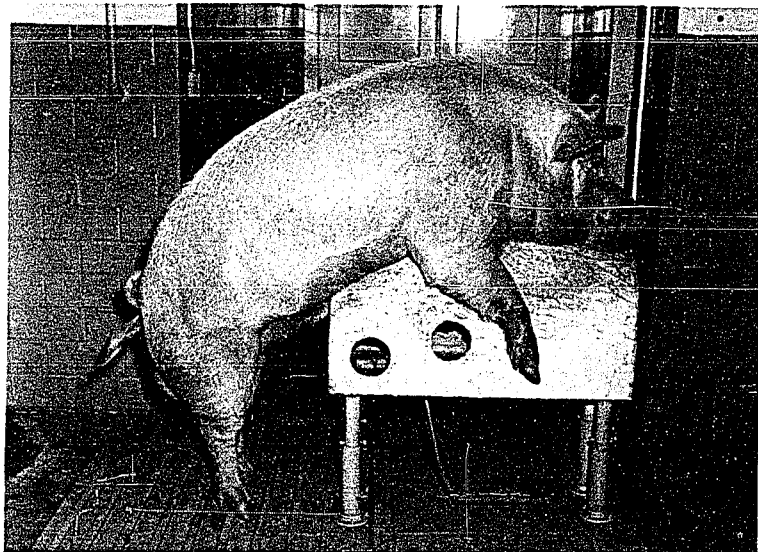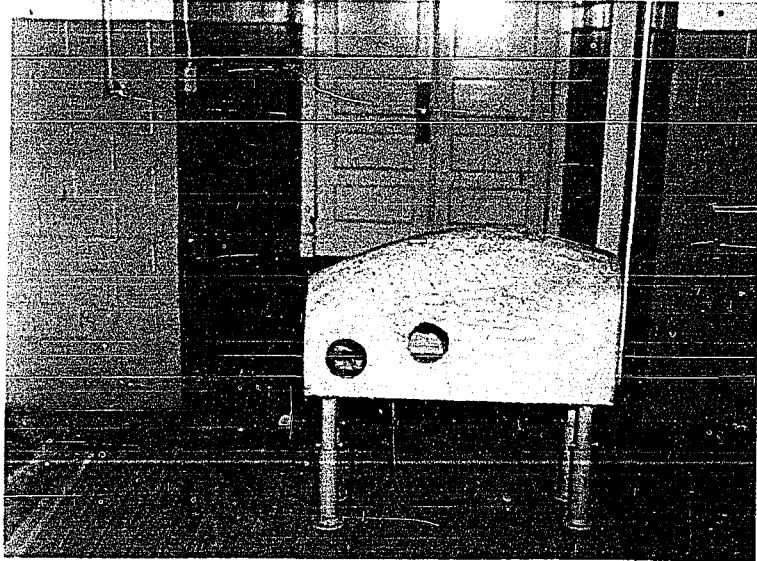
Figure 8. Insemination equipment - 50 cc. glass syringe
and modified bovine plastic inseminating pipette
used for inseminating sows

Figure 9. Insemination equipment - pressure bottle and
pipette
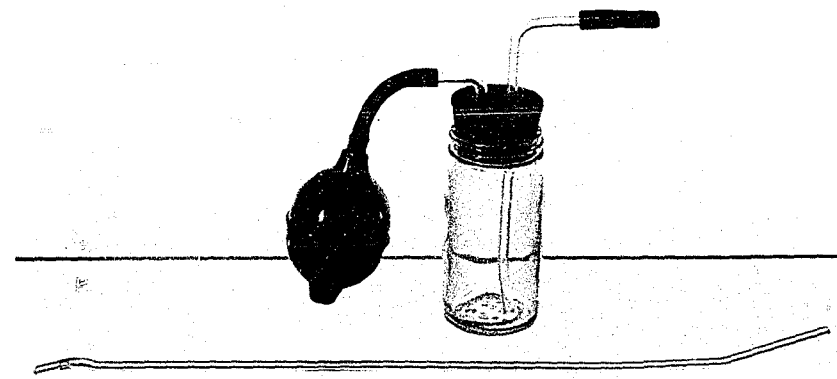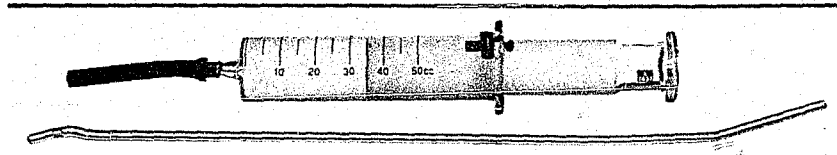
Figure 10. Fifty cc. glass syringe being used to inseminate
           sow

Figure 11. Pressure bottle being used to inseminate sow
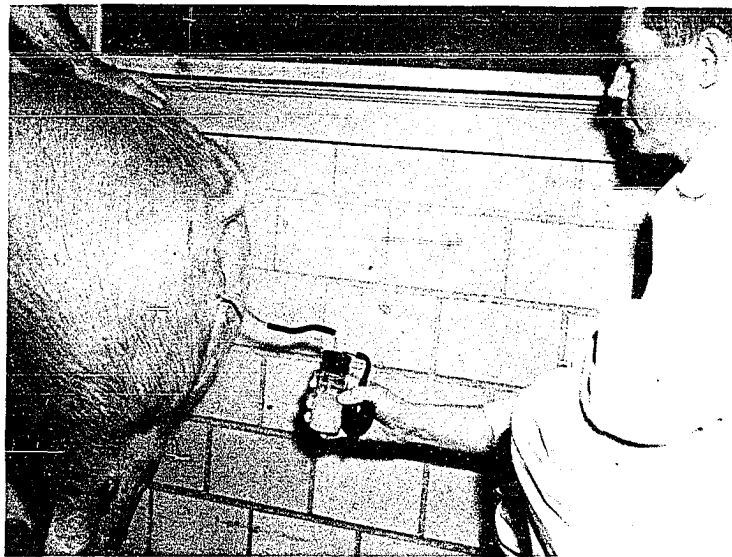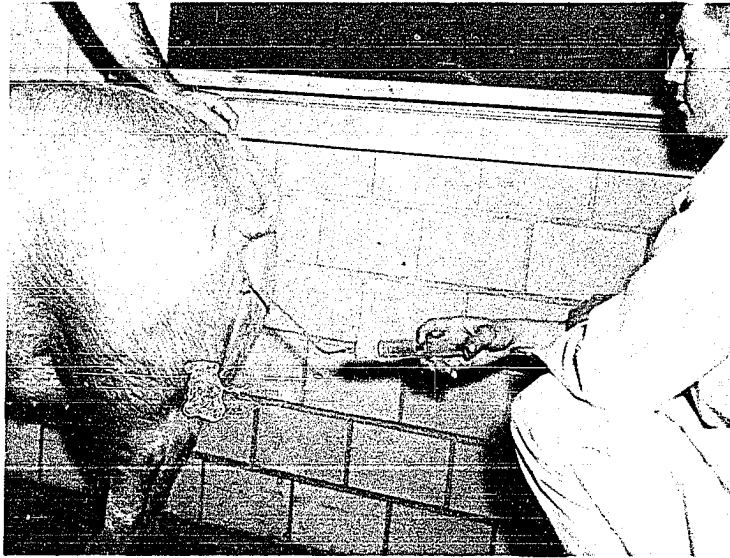
Figure 12. Schematic drawing of genital system of sow to
show locations from which tissue blocks were
taken for sectioning

ovary

oviduct

uterine horn

uterine body

cervix
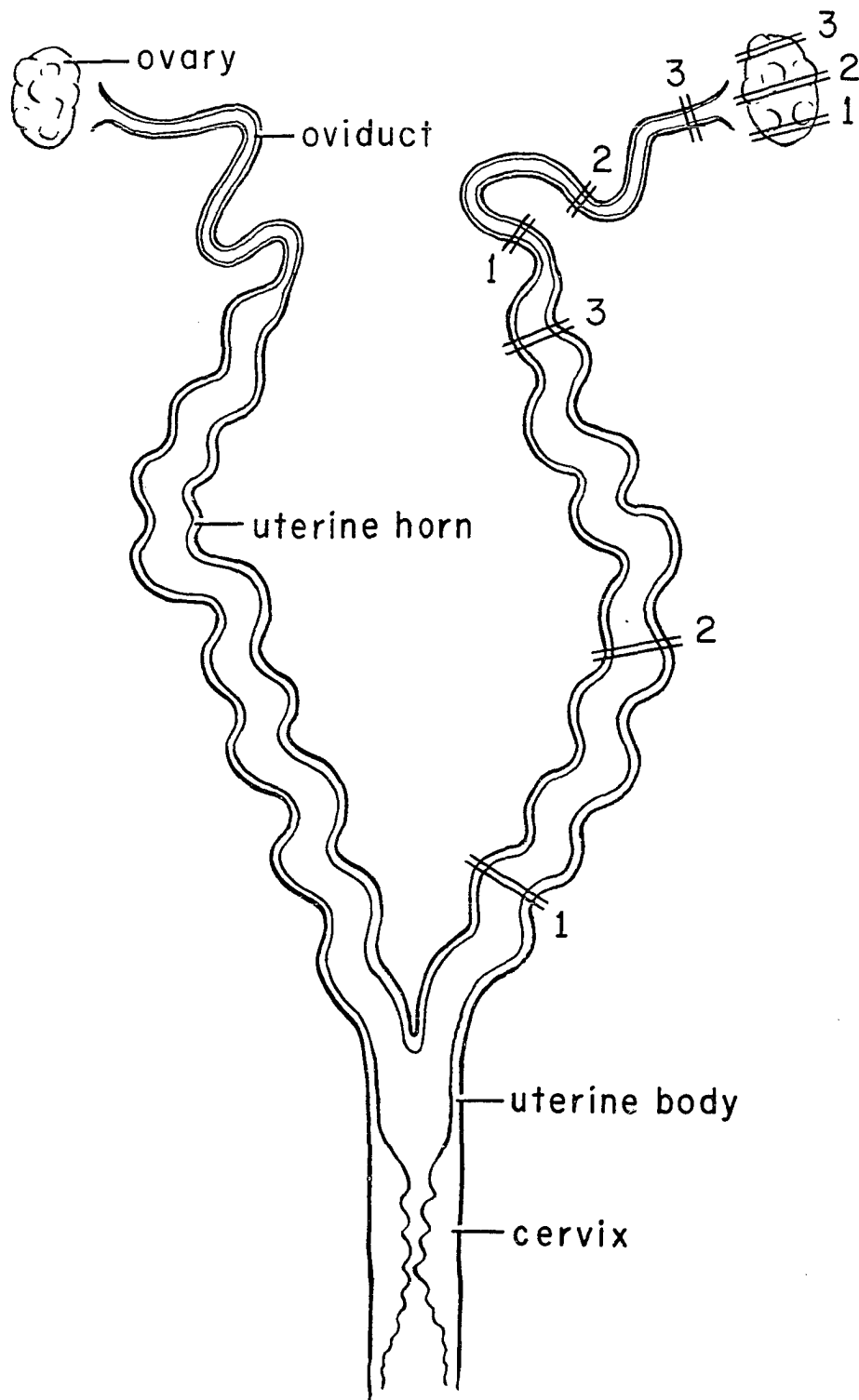
Figure 13. Schematic drawing of genital tract of boar to
show location from which tissue blocks were
taken for sectioning

vas deferens

tail of
epididymis

head of
epididymis

testis

mediastinum
testis
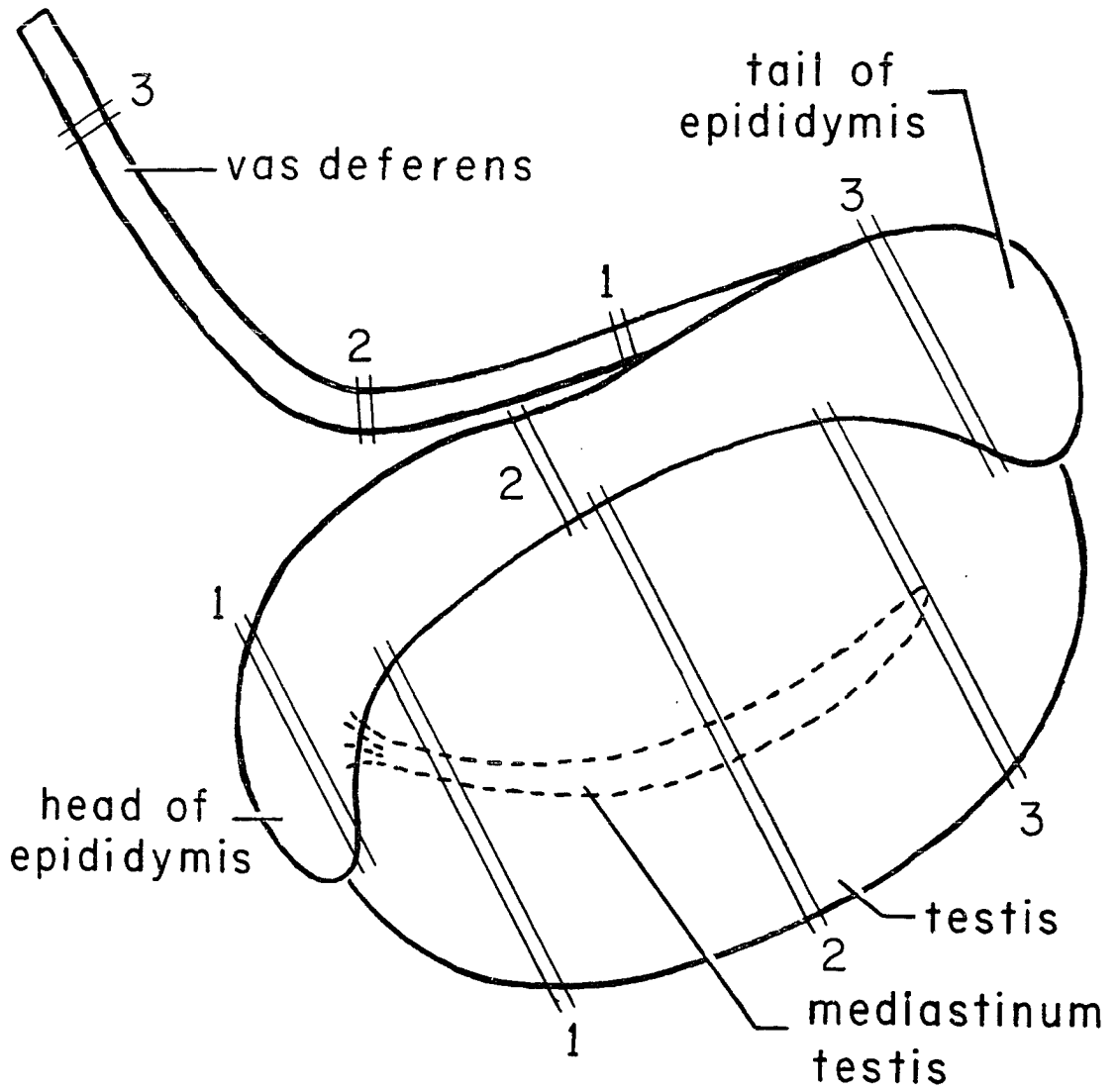
Figure 14. Schematic drawing to show stages of spermio-
genesis as followed in PAS preparations

Golgi phase

1. Early spermatid
2. Granules become visible in the Golgi area
3. Granules coalesce to form acrosome granule
4. Acrosomic vesicle develops

Cap phase

5. Vesicle collapses and granule starts
    to form cap
6. Cap starts to cover nucleus
7. Cap continues to spread
8. Cap covers half of nucleus
9. Cap covers more than half of nucleus

Acrosome phase

10. Tail is evident, nucleus is no longer
     covered by cytoplasm
11. Tail differentiates further
12. Manchette evident, head takes on typical
     sperm shape
13. Acrosome is differentiated

Maturation phase

14. Cytoplasm disappears from spermatid
15. Manchette area becomes narrower
16. Further loss of cytoplasm
17a. Mature spermatozoa ready to be released
17b. Lateral view of mature spermatozoa as
     found free in seminiferous tubules
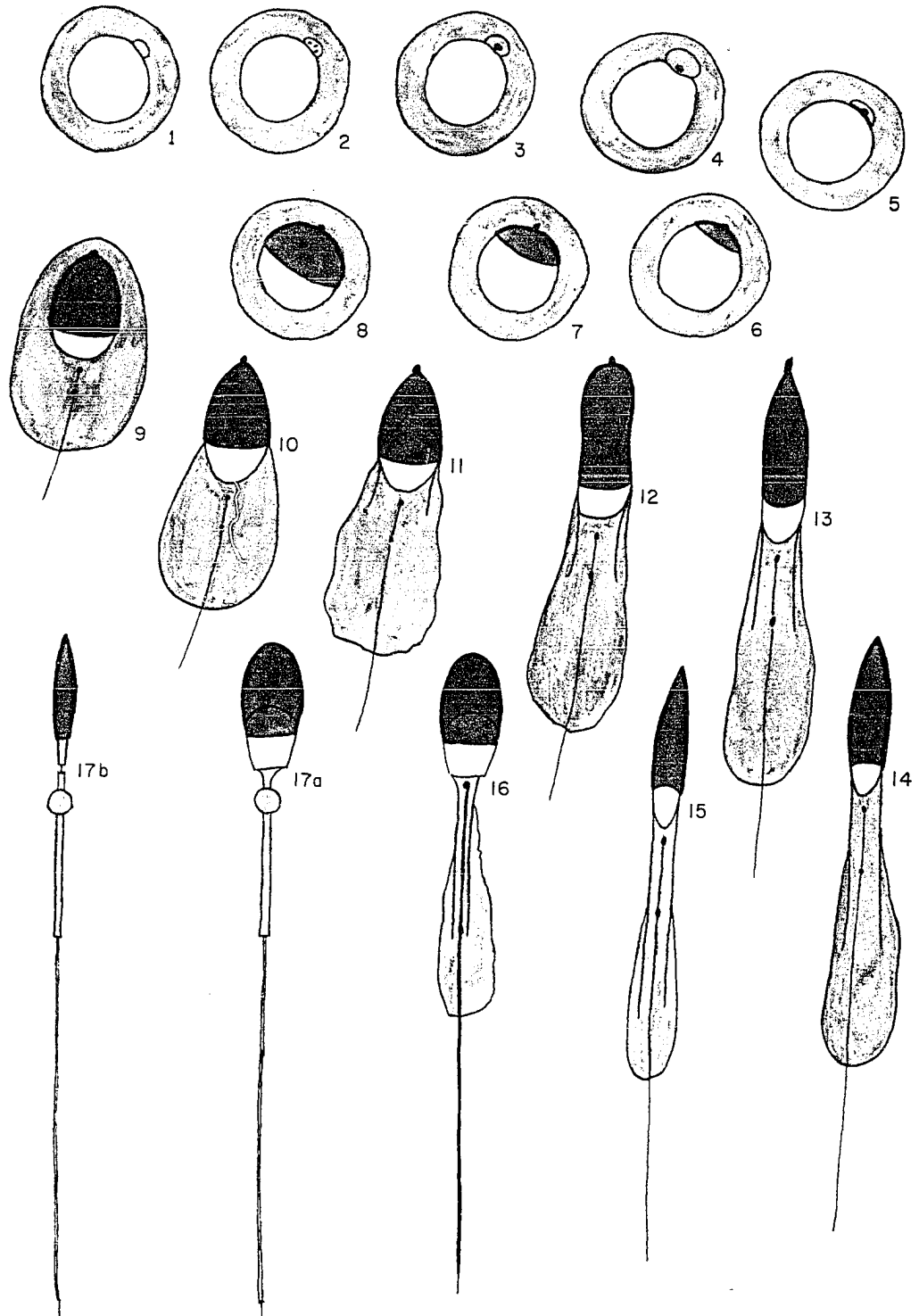
Figure 15.   Section of testicle from boar three, fixed in
             Zenkers formol and stained with periodic acid
             Schiff technique following diastase digestion
             895X

             a.   Spermatid in Golgi phase with achrosomic
                  vesicle
             b.   Spermatid in maturation phase

Figure 16.   Section of testicle from boar three, fixed in
             Zenkers formol and stained with periodic acid
             Schiff technique following diastase digestion
             895X

             a.   Spermatid in cap phase
             b.   Spermatid in maturation phase
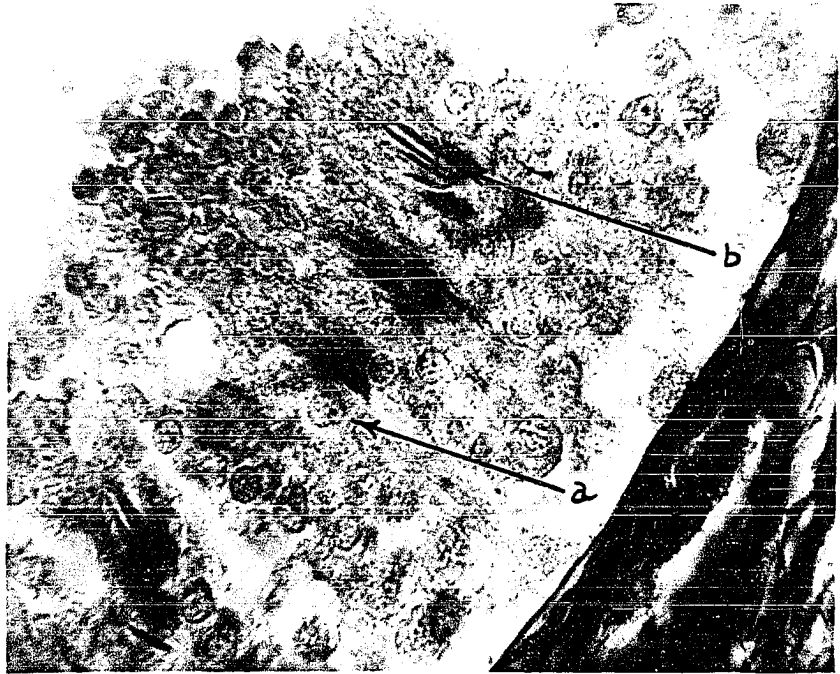
Figure 17.   Section of testicle from boar three, fixed in
              Zenkers formol and stained with periodic acid
              Schiff technique following diastase digestion
              895X

              a.   Spermatids in cap phase
              b.   Spermatids in maturation phase

Figure 18.   Section of testicle from boar three, fixed in
              Zenkers formol and stained with periodic acid
              Schiff technique following diastase digestion
              895X

              a.   Spermatid in cap phase
              b.   Spermatids in maturation phase
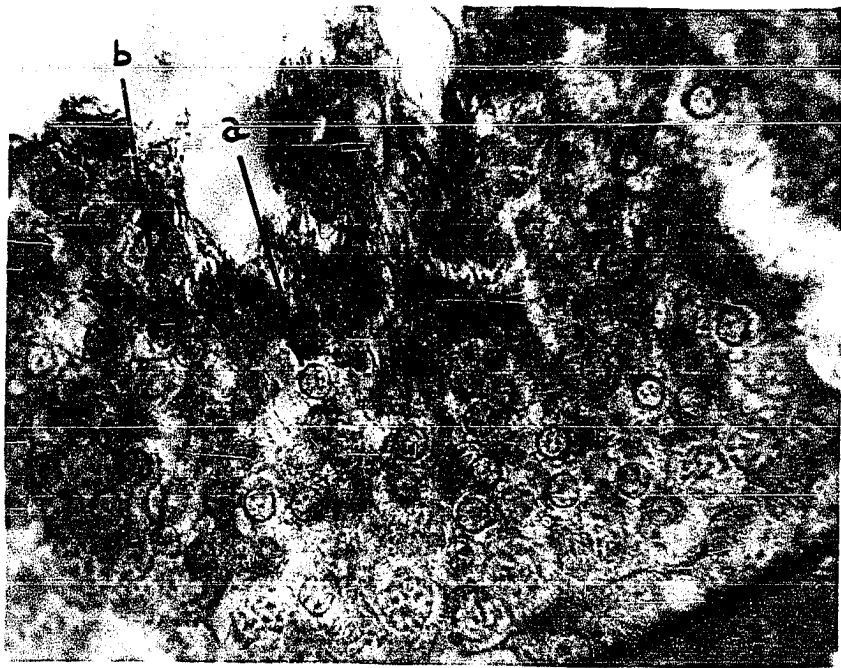
Figure 19. Section of testicle from boar three fixed in
Zenkers formol and stained with periodic acid
Schiff technique following diastase digestion
895X

      a. Spermatid in achrosome phase
      b. Interstitial cell

29

30

31

32

33

34

35

36

37
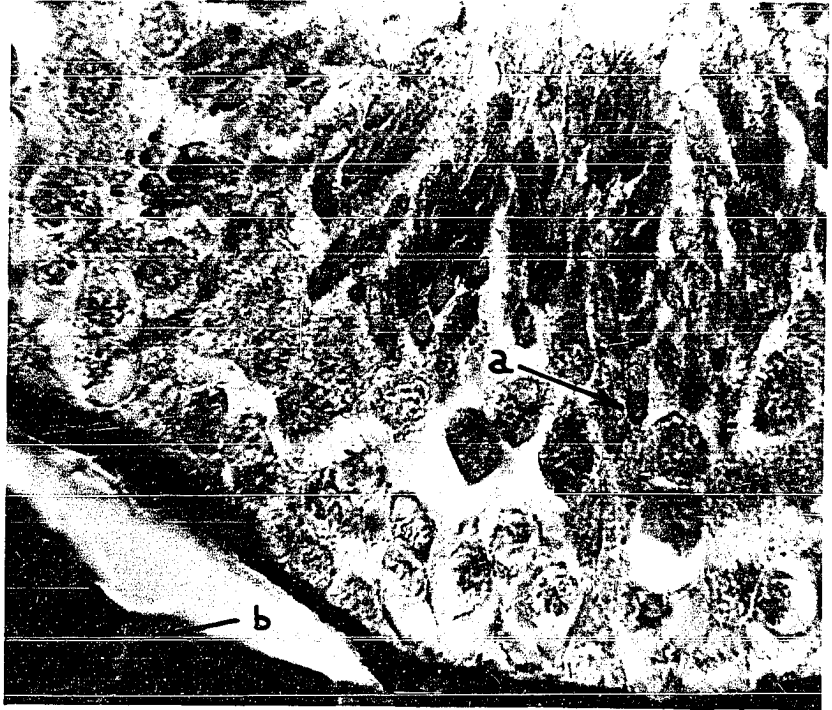
Figure 38. Section of testicle from boar five area three, fixed in acetone and incubated with acid phosphatase technique 48 hours   495X

    a. Late spermatids
    b. Area of reaction where spermatid heads are clumped
    c. Basement membrane reaction
    d. Cytoplasm of Sertoli cell extends down to basement membrane

Figure 39. Section of testicle from boar six area two, fixed in 80 per cent alcohol and incubated with alkaline phosphatase technique four hours   495X

    a. Late spermatids
    b. Early spermatids
    c. Area of reaction where spermatid heads are clumped
    d. Basement membrane reaction
    e. Leydig cells react strongly
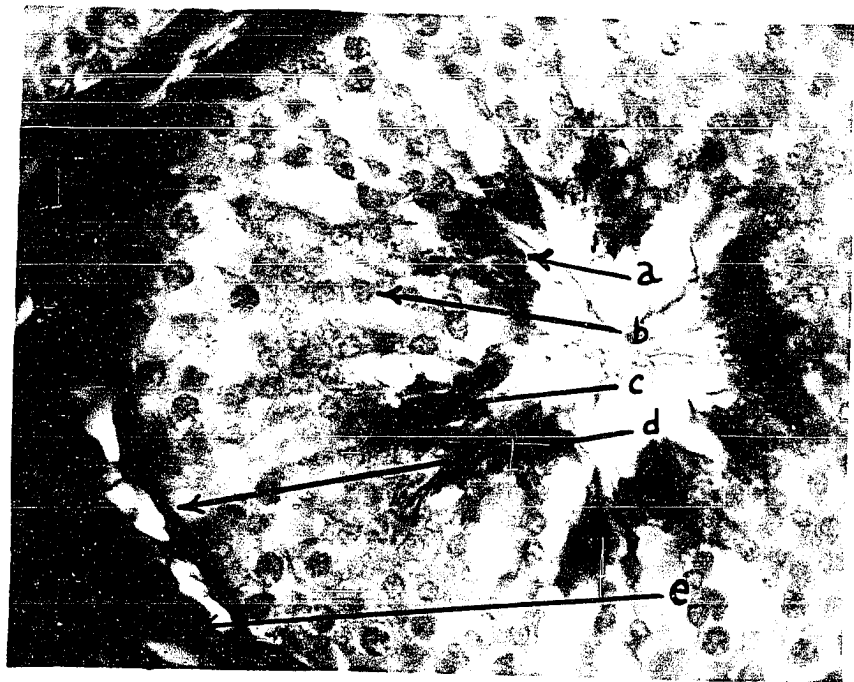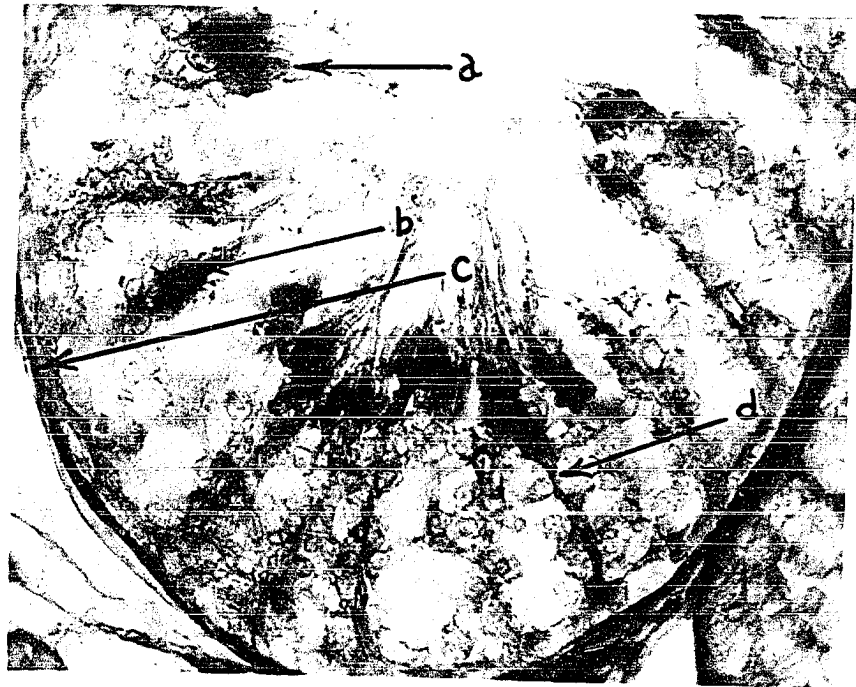
Figure 40.   Section of testicle from boar three, area two, fixed in Zenkers formol and stained with the periodic acid Schiff reaction following diastase digestion   495X

        a.   Spermatids in cap phase
        b.   Interstitial cell

Figure 41.   Section of testicle from boar three, area two, fixed in Zenkers formol and stained with periodic acid Schiff reaction following diastase digestion   495X

        a.   Spermatids in achrosome phase
        b.   Basement membrane
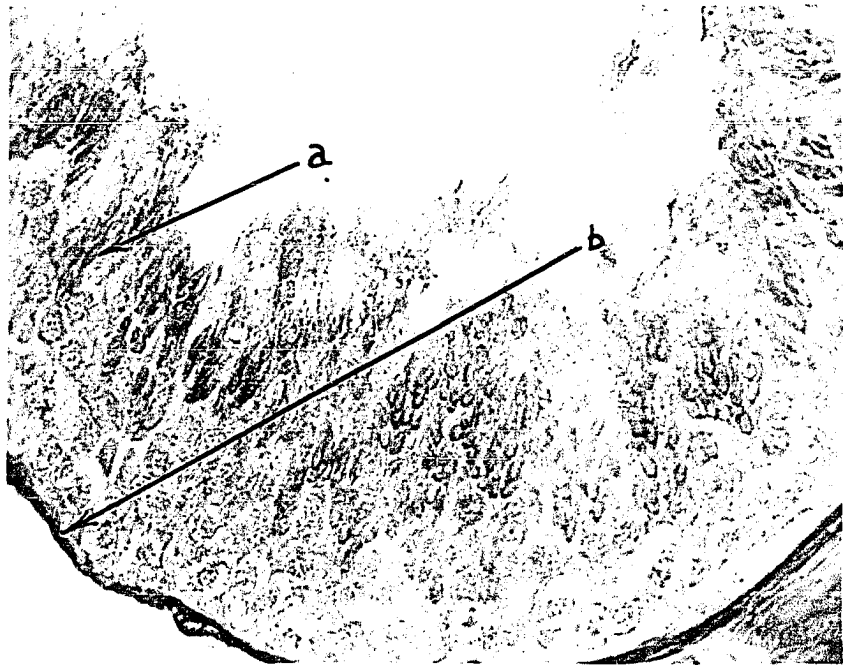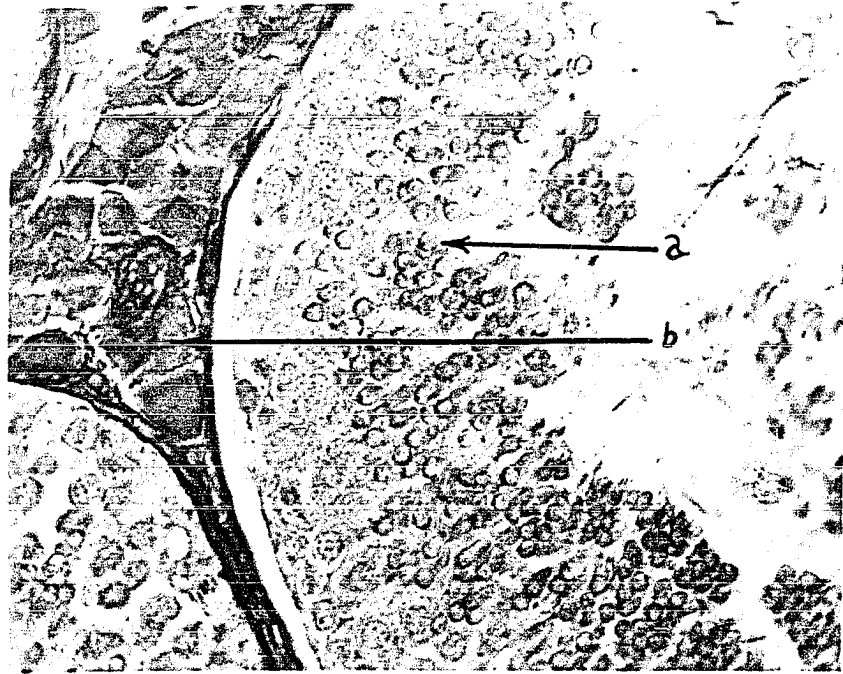
Figure 42. Section of testicle from boar three, area two,
fixed in Zenkers formol and stained with
periodic acid Schiff reaction following diastase
digestion   495X

a. Spermatids in maturation phase
b. Spermatids in Golgi phase
c. Empty vacuole

Figure 43. Section of testicle from boar two, area three,
fixed in Rossman's fixative and treated with
Bauer-Feulgen technique for demonstration of
glycogen

a. Glycogen granules in Sertoli cell
b. Glycogen granules in basement membrane
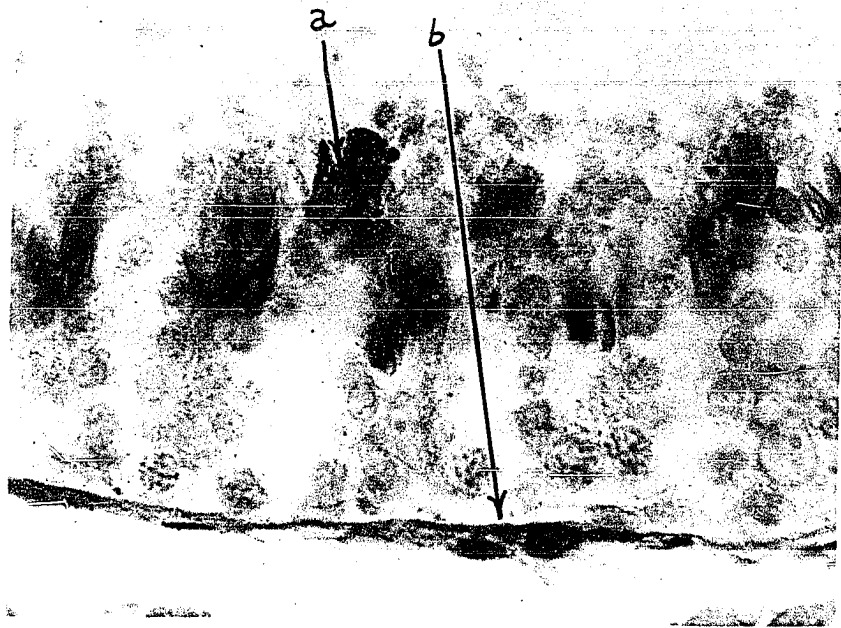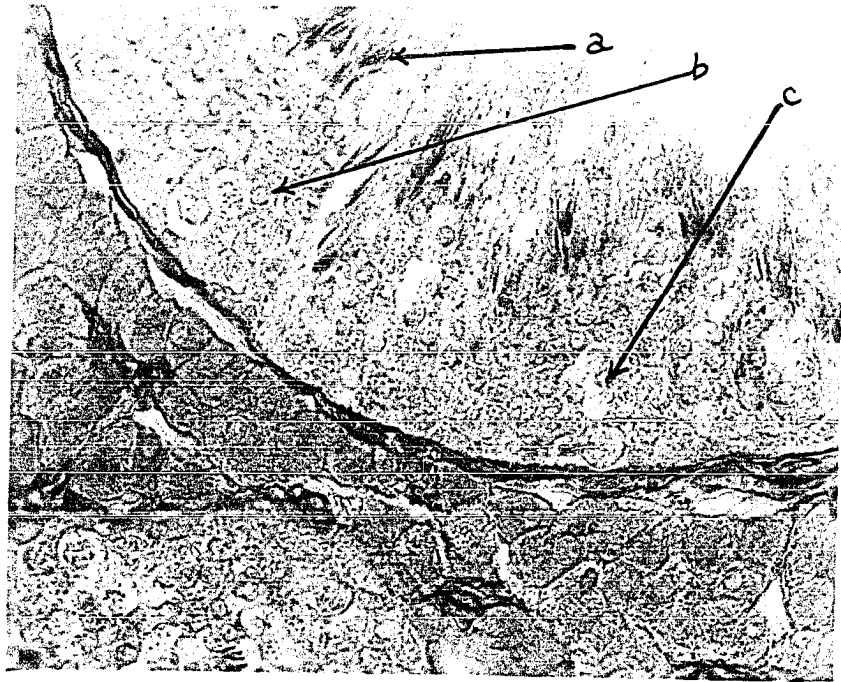
Figure 44.   Section of testicle from boar six fixed in
cadmium calcium formol and stained with oil
red O   190X

        a.   Lipid droplets around periphery of
seminiferous tubules
        b.   Interstitial cells

Figure 45.   Section of testicle from boar six fixed in
cadmium calcium formol and stained with oil
red O   495X

        a.   Lipid droplets around periphery of
seminiferous tubules
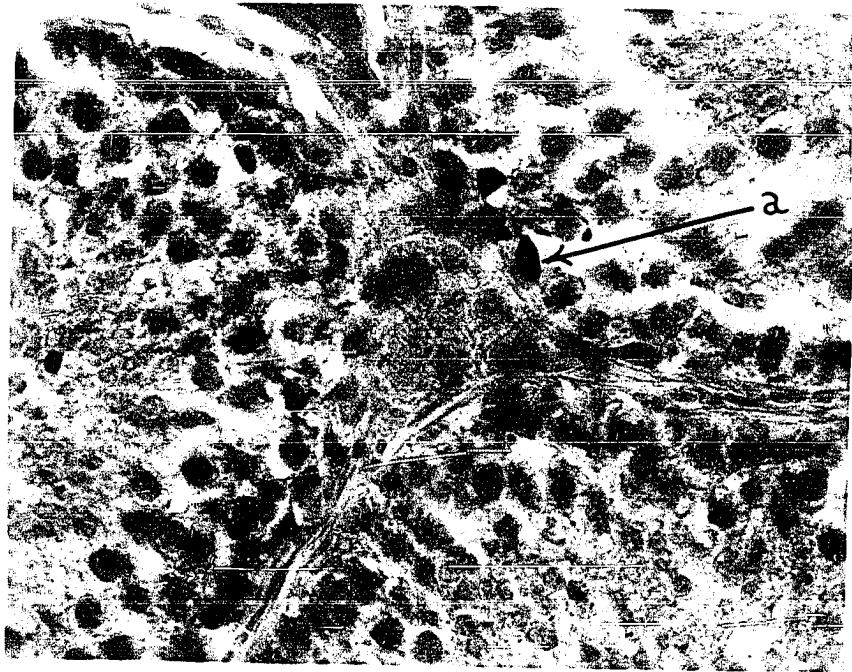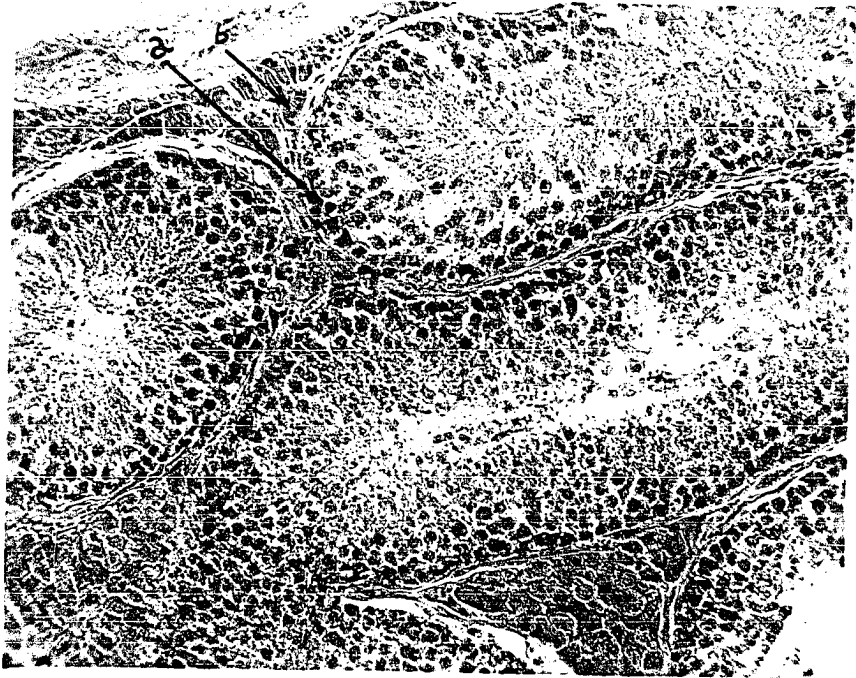
Figure 46.   Section of testicle from boar two, area three,
             fixed in Rossman's fluid and impregnated with
             Gomori's silver technique   495X

             a.   Basement membrane
             b.   Reticular fibers
             c.   Interstitial cells
             d.   Primary spermatocyte

Figure 47.   Section of testicle from boar two, area three,
             fixed in Rossman's fluid and impregnated with
             Gomori's silver technique   895X

             a.   Chromosomes in primary spermatocyte
             b.   Late spermatid
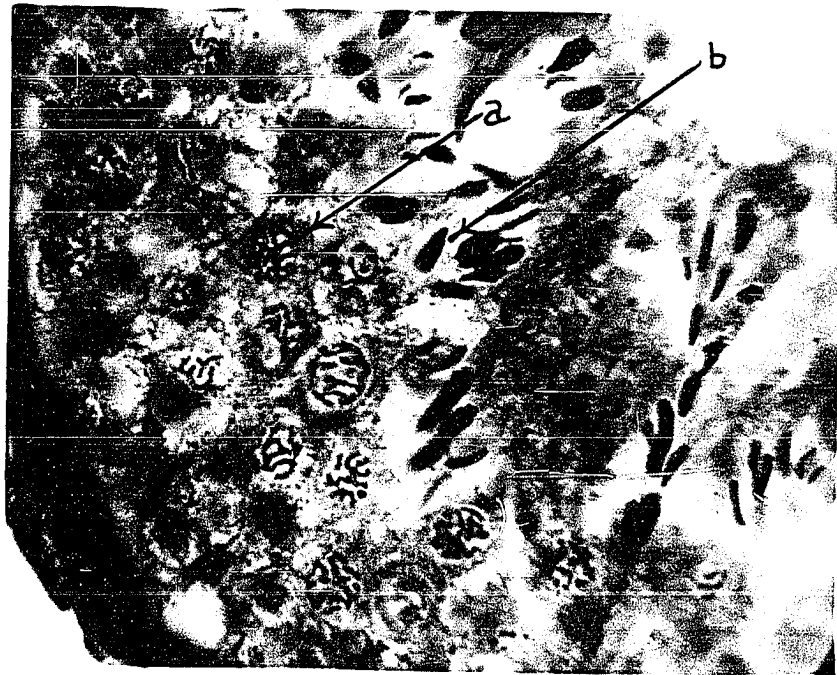
Figure 48.   Section of testicle from boar two, area three,
             fixed in Rossman's fluid and stained with
             hematoxylin and eosin   495X

        a.   Cytoplasmic droplets discarded from
             maturing spermatids

Figure 49.   Section of testicle from boar two, area three,
             fixed in Rossman's fluid and stained with
             hematoxylin and eosin   495X

        a.   Residual bodies
        b.   Empty vacuole
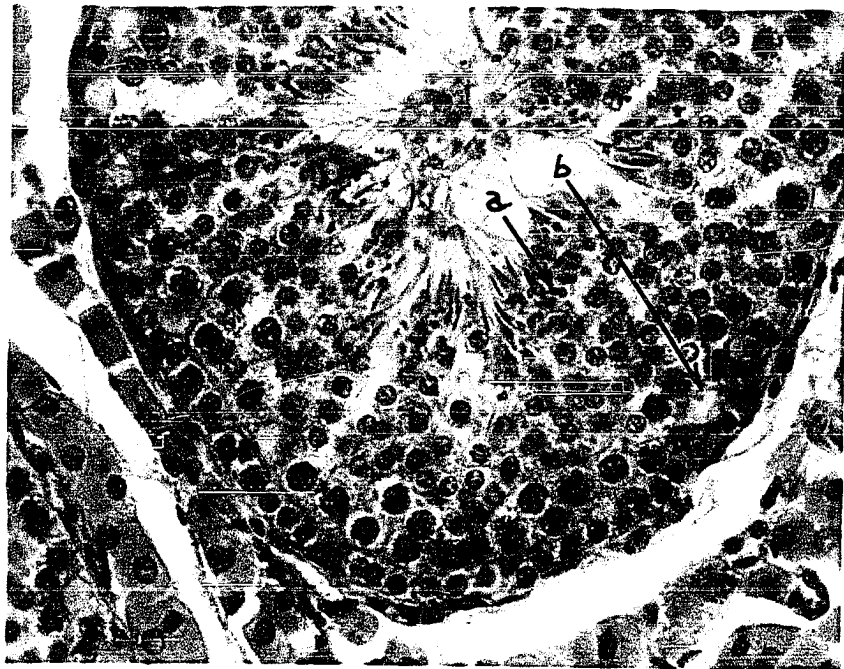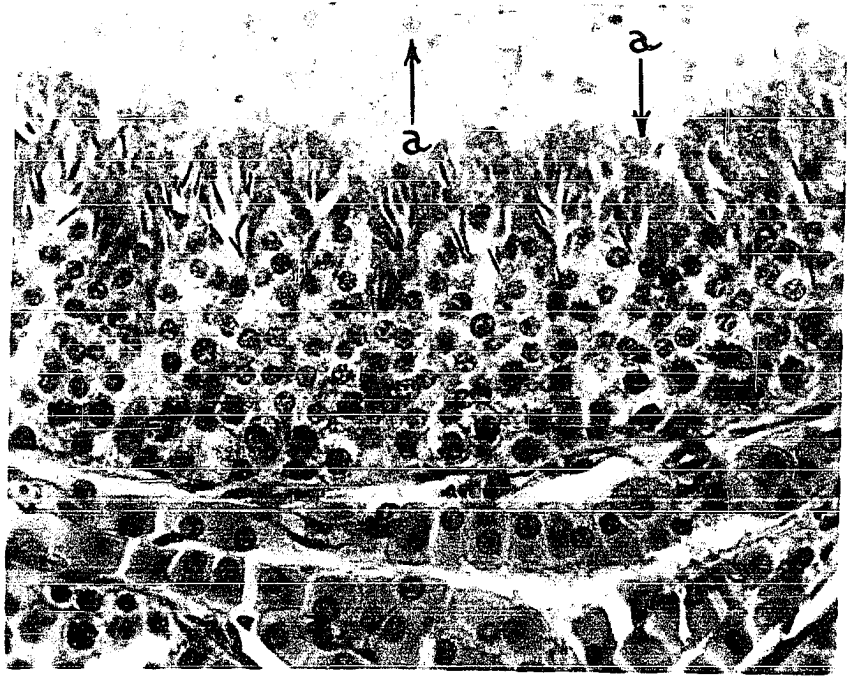
Figure 50.   Section of testicle from boar two, area three, fixed in Rossman's fluid and stained with Weigert's, Van Gieson's and Heidenhain's stain   495X

        a.   Spermatids in achrosome phase
        b.   Spermatocyte I

Figure 51.   Section of testicle from boar two, area three, fixed in Rossman's fluid and stained with Weigert's, Van Gieson's and Heidenhain's stain   495X

        a.   Early spermatids
        b.   Late spermatids in maturation phase
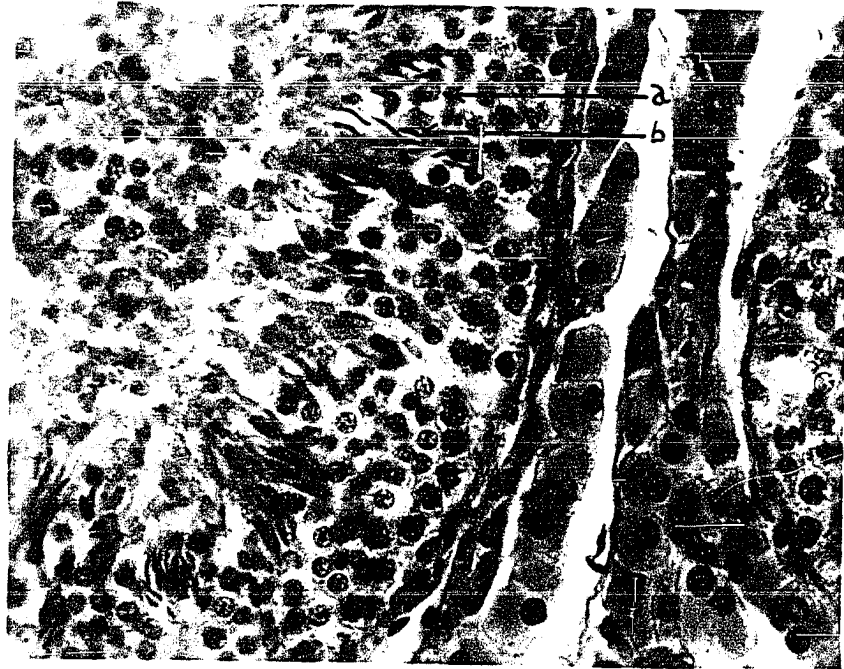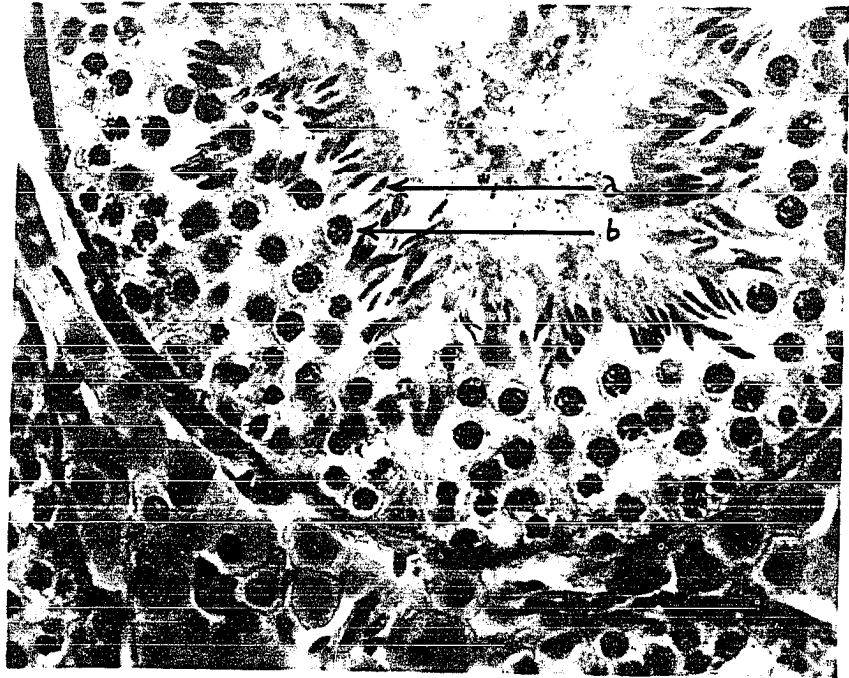
Figure 52. Section of head of epididymis from boar three
fixed in alcohol, acetic acid, and formalin
stained with tri-chrome stain   495X

     a. Stereocilia
     b. Vacuoles

Figure 53. Section of body of epididymis from boar six
fixed in 10 per cent formalin and stained with
hematoxylin and eosin   495X

     a. Stereocilia
     b. Spermatozoa in lumen of tubule
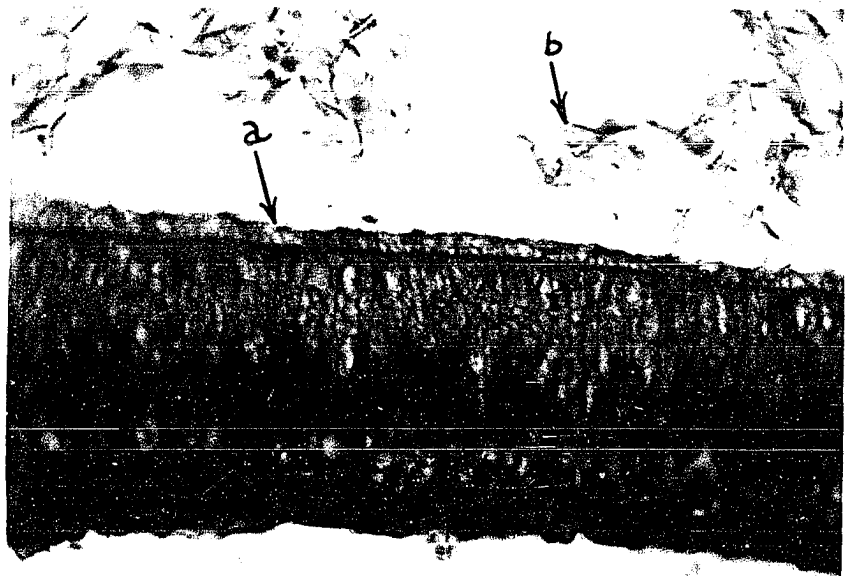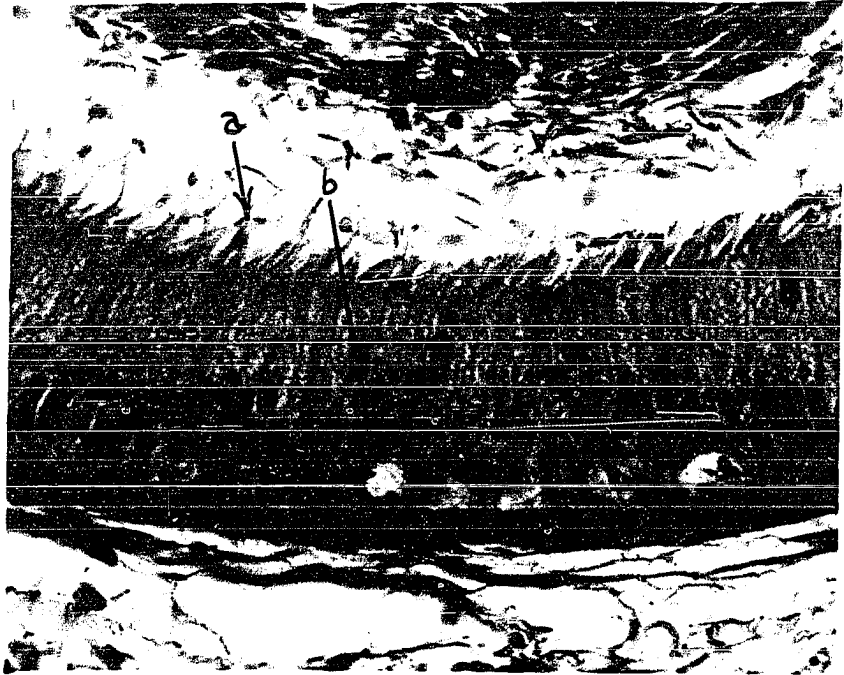
Figure 54. Section of head of epididymis from boar two
fixed in cold acetone and stained with acid
phosphatase technique 24 hours   495X

    a. Stereocilia and distal third of cytoplasm
reacts
    b. Basement membrane and capillaries react

Figure 55. Section of body of epididymis from boar five
fixed in cold 80 per cent alcohol and stained
with alkaline phosphatase technique four
hours   495X

    a. Stereocilia and distal half of cytoplasm
react very strongly
    b. Basement membrane reaction
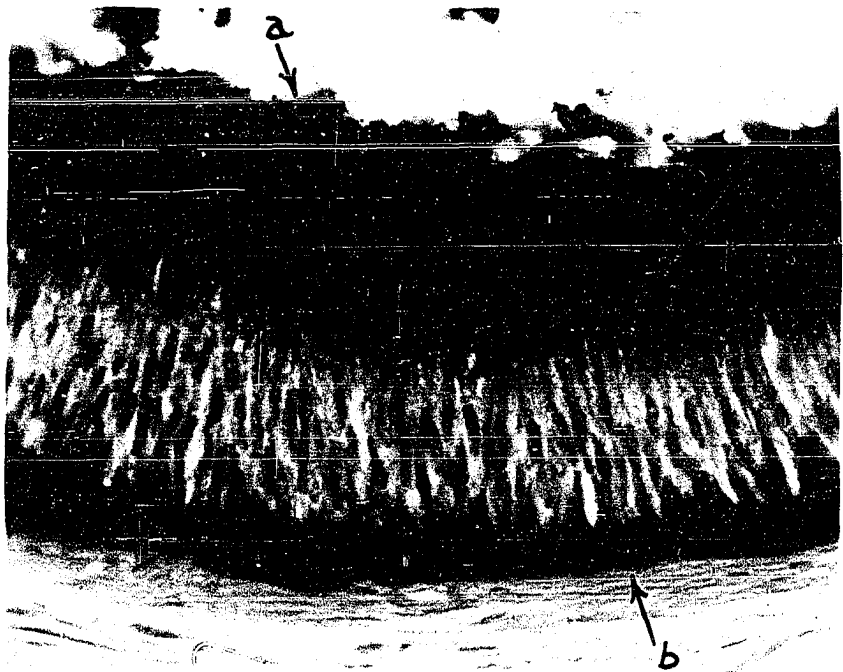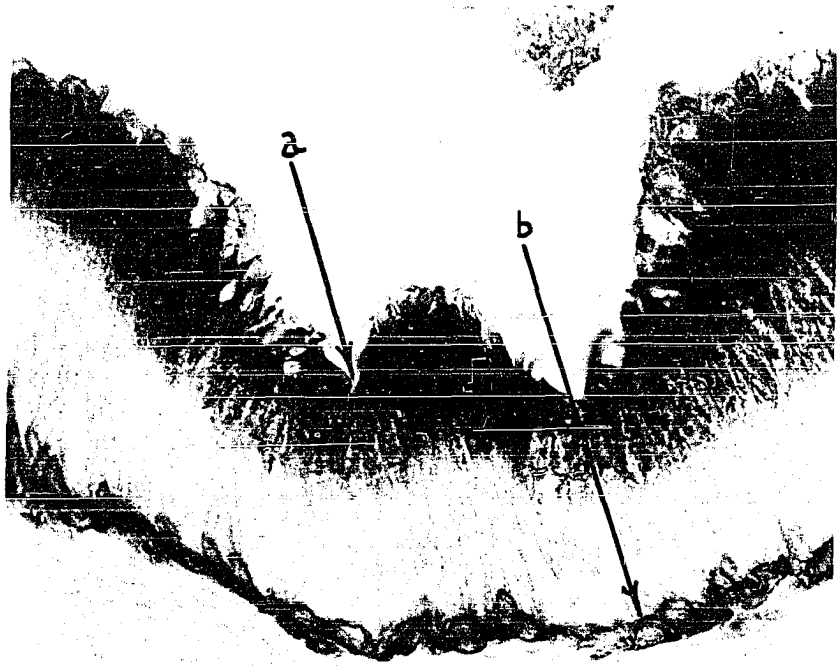
Figure 56. Section of head of epididymis from boar five
fixed in Rossman's fluid and stained with
Bauer-Feulgen technique for glycogen   96X

a. Cross section of tubule of epididymis
b. Glycogen reaction in vacuole of epididymis

Figure 57. Same section as Figure 56   495X

a. Positive reaction for glycogen in vacuoles
at the base of the epithelial cells
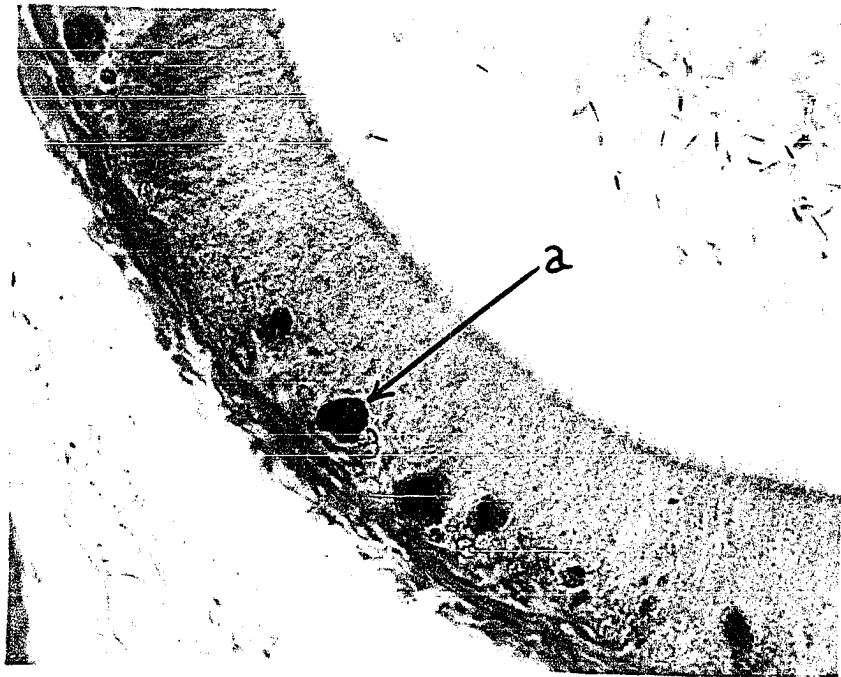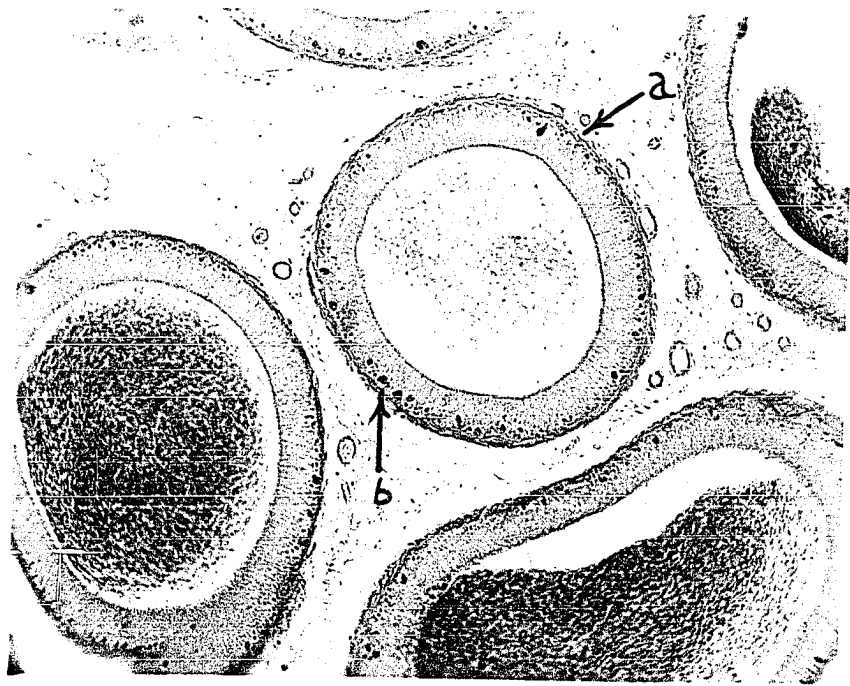
Figure 58.   Section of tail of epididymis of boar three
             fixed in 10 per cent formalin and stained with
             hematoxylin and eosin   96X

             a.   Undulating surface of epithelium
             b.   Thick connective tissue layer

Figure 59.   Section of tail of epididymis of boar two fixed
             in Rossman's fluid and stained with Bauer-
             Feulgen technique for glycogen   495X

             a.   Glycogen granules in cytoplasm of epithelial
                  cells of epididymis
             b.   Stereocilia
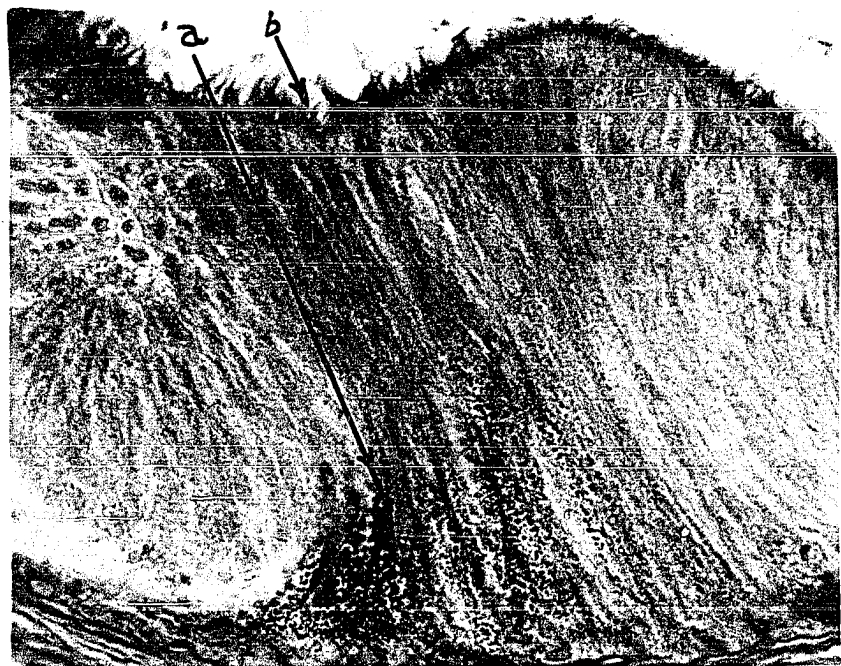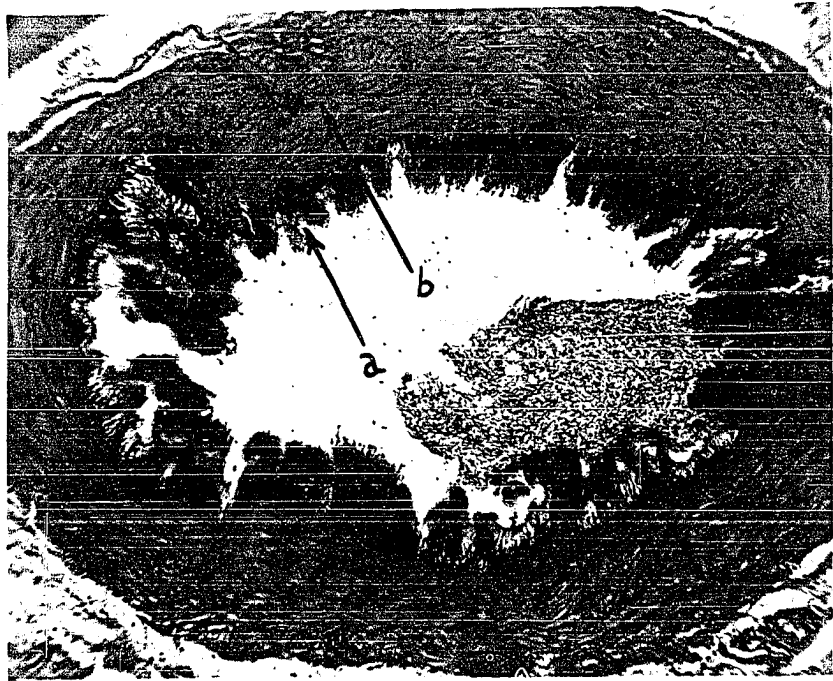
Figure 60.   Section of body of epididymis from boar six
             fixed in cadmium calcium formol and stained
             with oil red O   190X

             a.   Lipid secretion droplets
             b.   Spermatozoa in lumen

Figure 61.   Same section as Figure 60   495X

             a.   Lipid secretion droplets
             b.   Capillaries
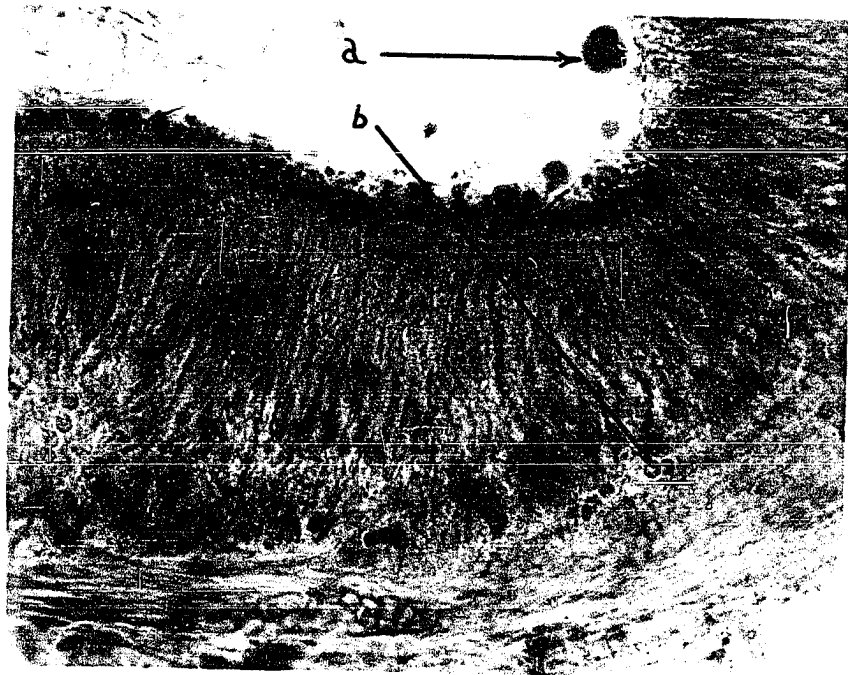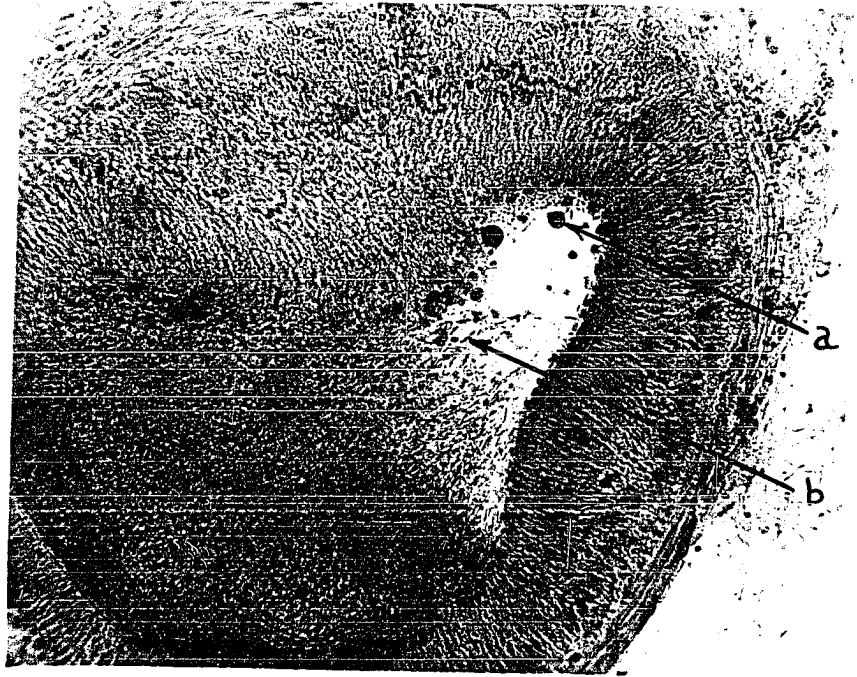
Figure 62.   Section of vas deferens area three from boar
             · six fixed in cold acetone and stained with
             acid phosphatase technique 24 hours   495X

             a. Reaction on surface of epithelial cells
             b. Basement membrane and associated capil-
                 laries show a reaction

Figure 63.   Section of vas deferens area three from boar
             three fixed in chilled 80 per cent alcohol
             and treated with alkaline phosphatase technique
             four hours   495X

             a. Positive reaction on surface of epithelial
                 cells
             b. Basement membrane and associated capillaries
                 show a reaction
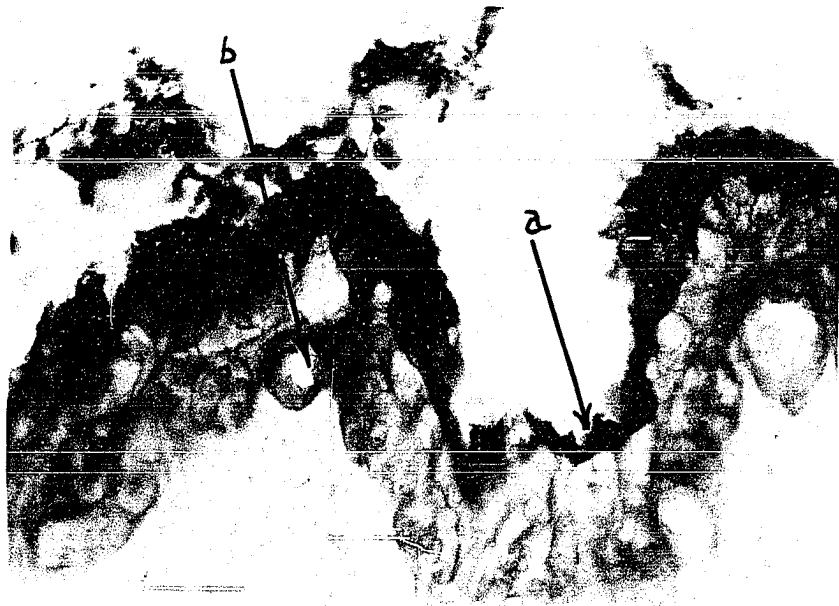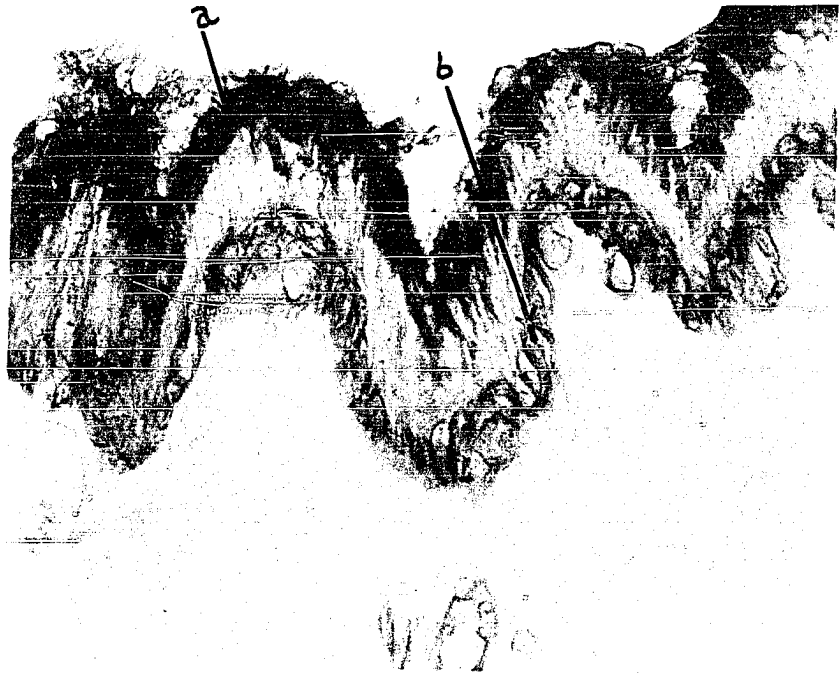
Figure 64.   Section of vas deferens area two from boar
             five fixed in 10 per cent formalin and
             stained with hematoxylin and eosin   47X

             a. Lumen
             b. Undulating epithelial lining

Figure 65.   Section of vas deferens area two from boar
             six fixed in Rossman's fluid and stained with
             hematoxylin and eosin   190X

             a. Lumen containing sperm
             b. Epithelial lining
             c. Muscular fibrous outer tunic
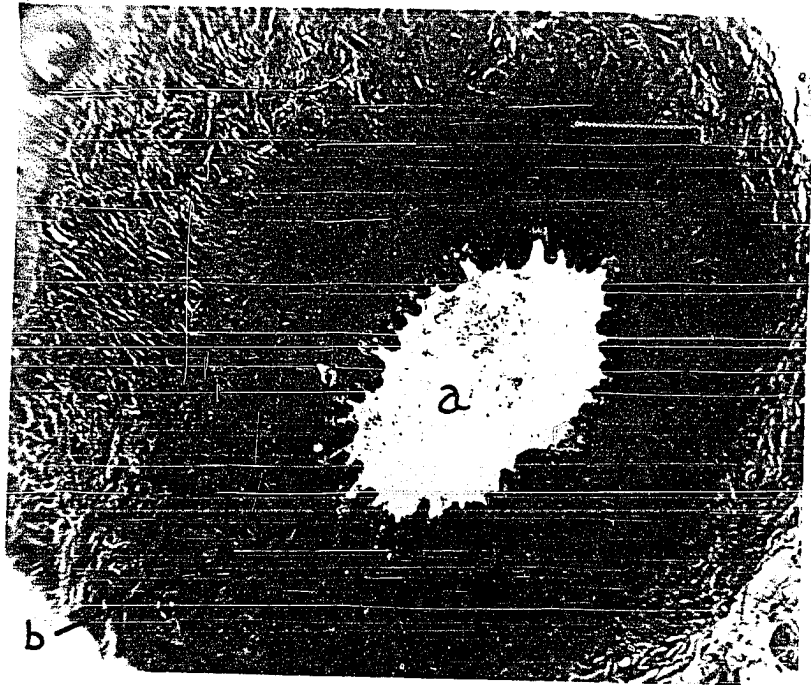
Figure 66. Section of uterus area two from sow five fixed in Rossman's fluid and stained with hematoxylin and eosin 96X

    a. Surface epithelium
    b. Blood vessels
    c. Edema spaces
    d. Uterine glands

Figure 67. Section of uterus area two from sow five fixed in Rossman's fluid and stained with hematoxylin and eosin 495X

    a. Vacuoles in epithelium near basement membrane
    b. Epithelium
    c. Connective tissue
    d. Capillaries
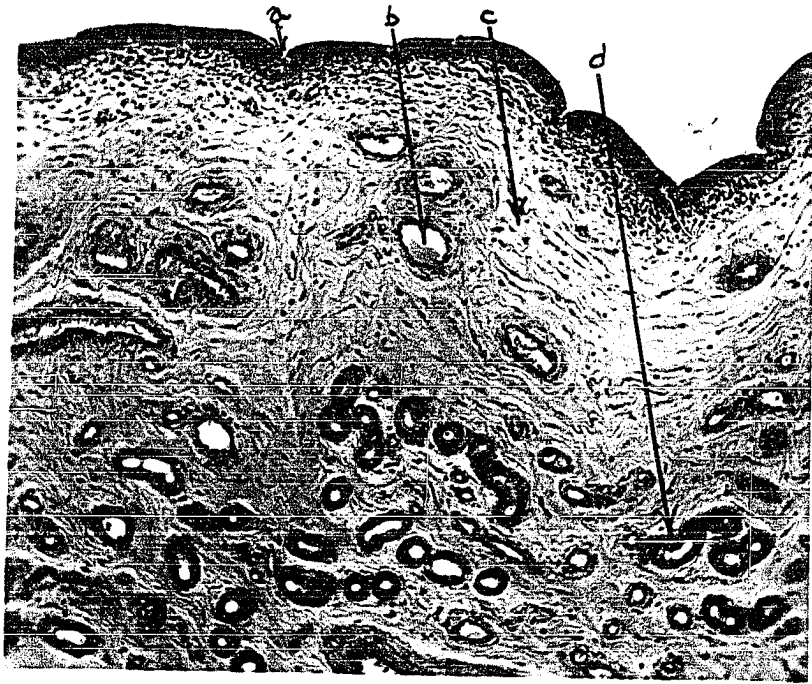
Figure 68. Section of uterus area two from sow four
fixed in Rossman's fluid and stained with
Weigert's, Van Gieson's, and Heidenhain's
stain   495X

   a. Mitotic figure anaphase
   b. Telophase

Figure 69. Section of uterus area two from sow four
fixed in Rossman's fluid and stained with
Weigert's, Van Gieson's, and Heidenhain's
stain   495X

   a. Columnar nature of epithelial cells can
       be observed
   b. Mitotic figure
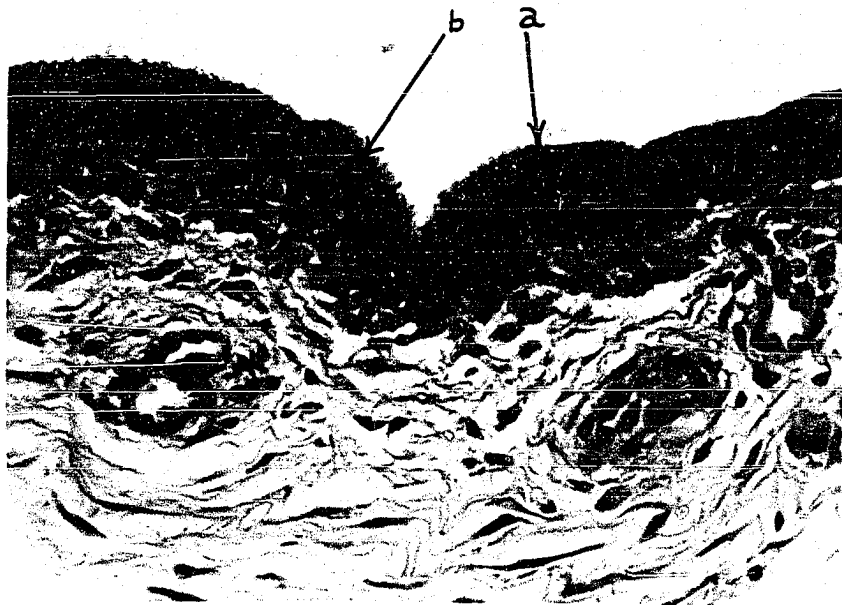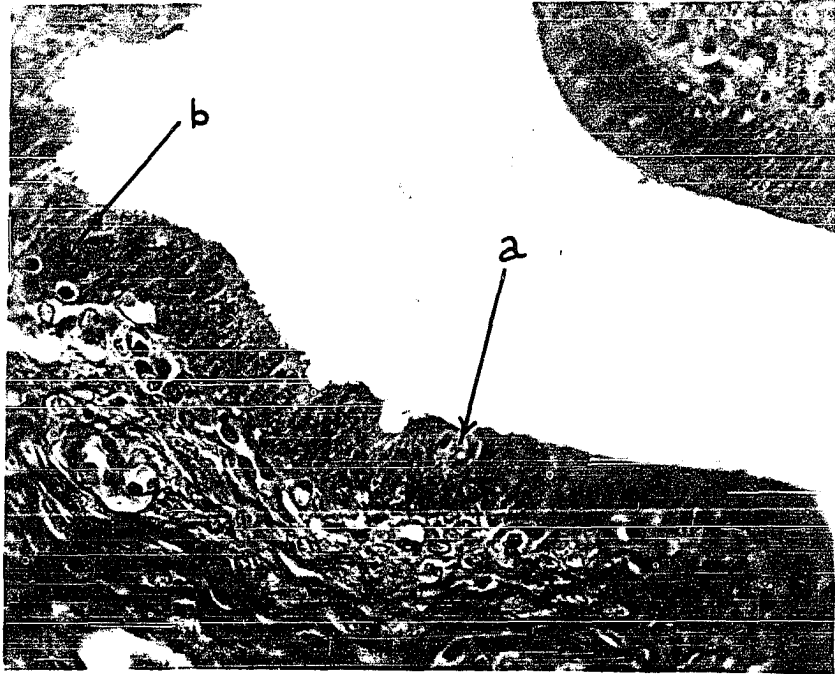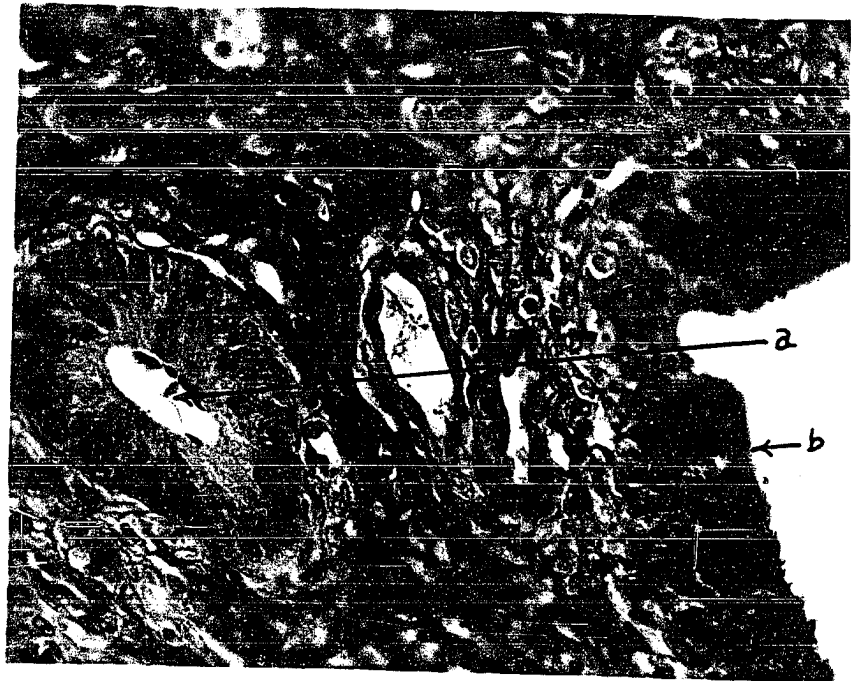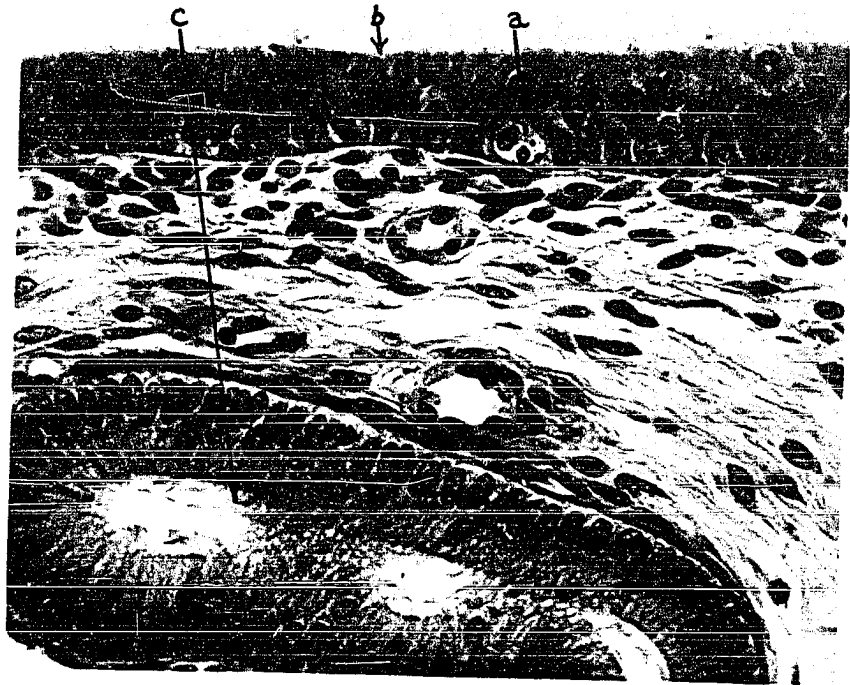
Figure 70.   Section of uterus area two from sow five fixed
in alcohol, acetic acid, and formalin and
stained with tri-chrome stain   495X

a.   Leucocytes under epithelium
b.   Leucocytes invading epithelium
c.   Leucocyte on surface of epithelium

Figure 71.   Section of uterus area one from sow four fixed
in 10 per cent formalin and stained with
hematoxylin and eosin   495X

a.   Leucocytes breaking through epithelium
making their way to the lumen of the uterus
b.   Capillary
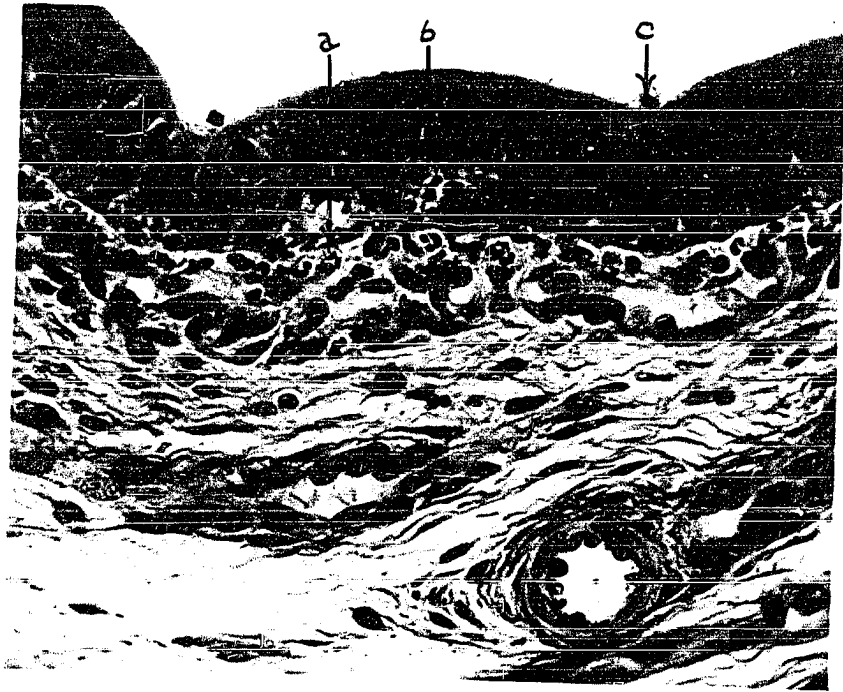
Figure 72.   Section of uterus area two from sow four fixed
             in alcohol, acetic acid, and formalin stained
             with tri-chrome stain   495X

             a. Vacuole in epithelium of uterus
             b. Non-ciliated surface epithelium
             c. Ciliated epithelium in neck of uterine
                 glands

Figure 73.   Section of uterus area two from sow four fixed
             in Rossman's fluid and stained with Weigert's,
             Van Gieson's, and Heidenhain's stain   495X

             a. Sperm in neck of uterine gland tubule
             b. Surface epithelium of uterus

Figure 74. Uterine glands - section of uterus area two sow
four fixed in alcohol, acetic acid, and
formalin stained with tri-chrome stain   495X

   a. Glandular epithelium

Figure 75. Uterine glands - section of uterus area one
sow four fixed in 10 per cent formalin and
stained with hematoxylin and eosin   895X

   a. Vacuole
   b. Glandular epithelium

Figure 76. Acid phosphatase reaction in uterus - section of uterus area three from sow two fixed in chilled acetone and incubated for 48 hours with acid phosphatase technique   495X

a. Surface of epithelial cells react
b. Capillaries react

Figure 77. Glycogen reaction in uterus - section of uterus area two from sow five fixed in Rossman's fluid and stained with the Bauer-Feulgen technique for glycogen   495X

a. Surface of epithelium shows reaction
b. Leucocytes show positive reaction
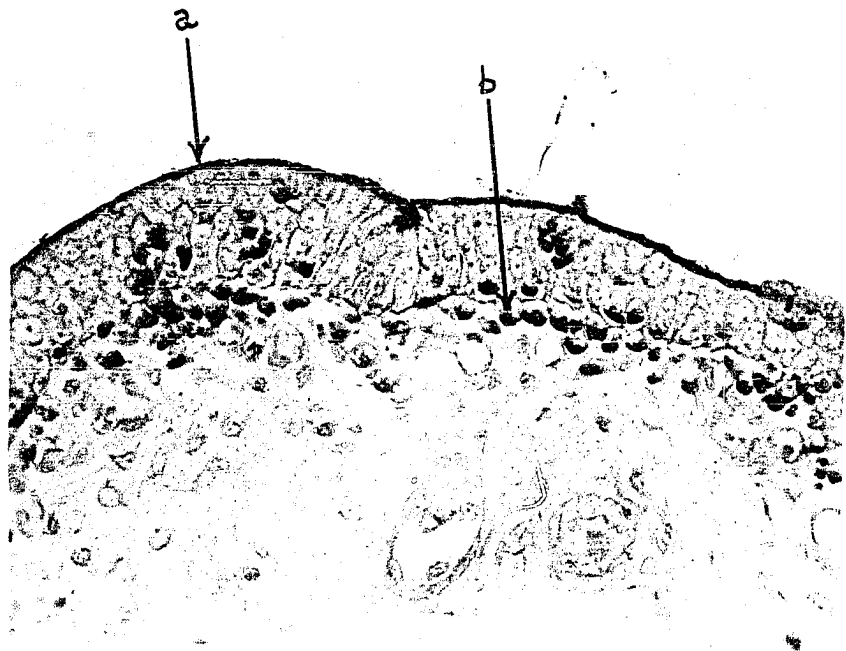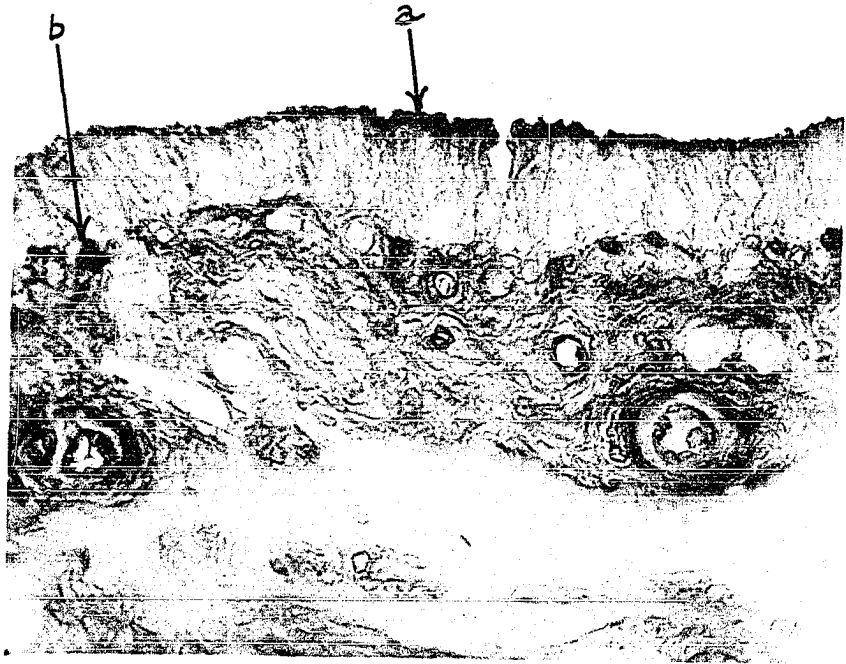
Figure 78. Alkaline phosphatase reaction in uterus - section
of uterus area one from sow five fixed in
chilled 80 per cent alcohol and incubated with
alkaline phosphatase technique four hours   495X

a. Surface of epithelium shows positive re-
    action
b. Capillaries associated with basement
    membrane are positive
c. Endothelium of larger blood vessels is
    also positive

Figure 79. Alkaline phosphatase reaction in uterine glands -
section of uterus area one from sow five fixed
in chilled 80 per cent alcohol and incubated
with alkaline phosphatase technique for four
hours   495X

a. Surface of glandular epithelium positive
    reaction
b. Basement membrane
c. Endothelium of blood vessel shows strong
    reaction

Figure 80. Reticular fibers in uterus - section of uterus
area two from sow five fixed in Rossman's
fluid and stained with Gomori's silver impreg-
nation technique   190X

    a. Reticular network under the uterine epi-
       thelium
    b. Collagenous fibers
    c. Uterine gland basement membrane composed
       of reticular fibers

Figure 81. Reticular fibers in uterus - section of uterus
area two from sow two fixed in Rossman's fluid
and stained with Gomori's silver impregnation
technique   495X

    a. Reticular network making up basement
       membrane of epithelium
    b. Capillary channels through reticular network
    c. Uterine gland tubule showing reticular
       fibers in basement membrane
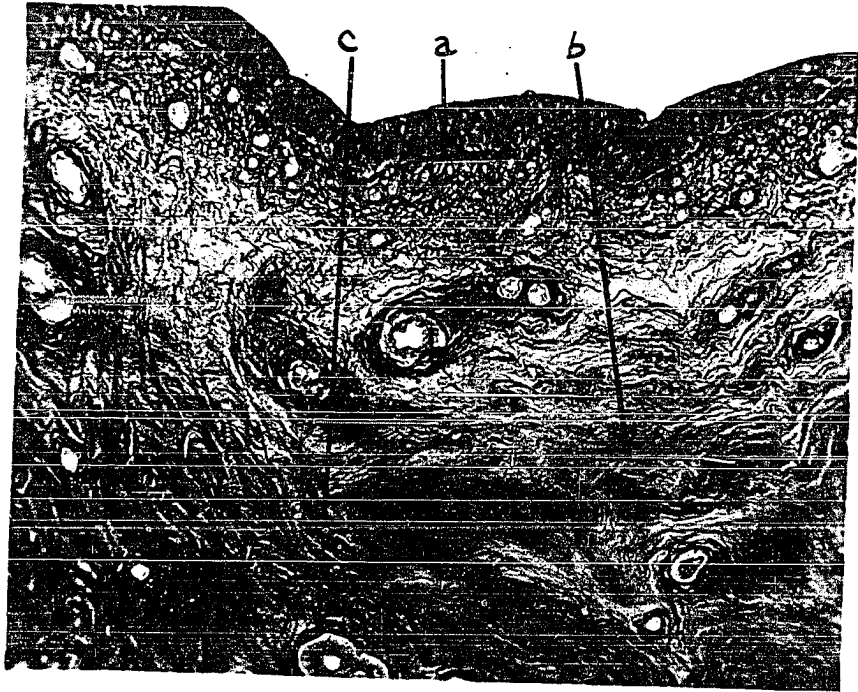
Figure 82.   Section of ampulla of oviduct area three from
             sow two fixed in 10 per cent formalin stained
             with hematoxylin and eosin   96X

             a.   Complex folds or villus-like projections
                  into the lumen
             b.   Outer muscular wall of oviduct
             c.   Epithelium of oviduct

Figure 83.   Section of oviduct area three from sow four
             fixed in alcohol, acetic acid, and formalin
             and stained with tri-chrome stain   895X

             a.   Ciliated epithelium
             b.   Secretion epithelium
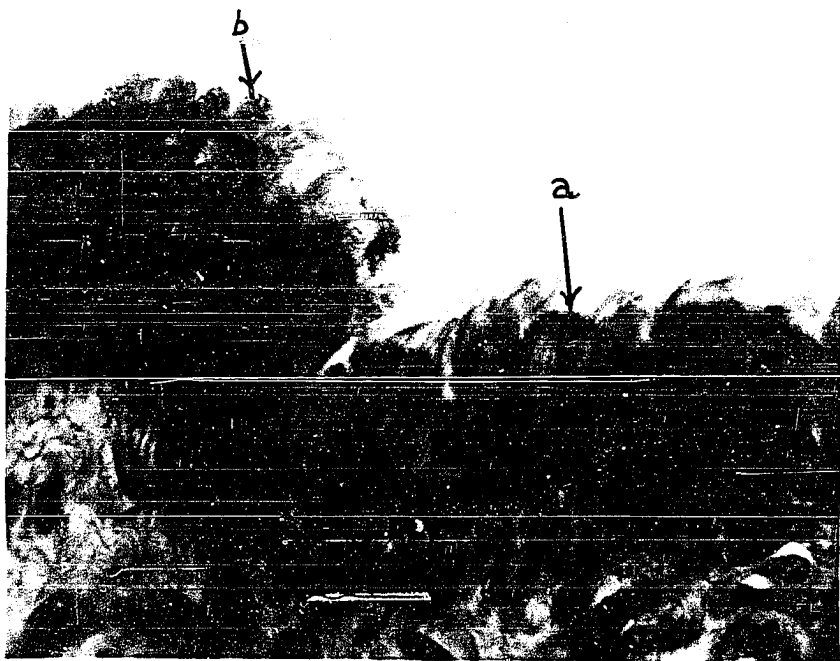
Figure 84. Alkaline phosphatase in isthmus of oviduct -
section of oviduct area one from sow four
fixed in chilled 80 per cent alcohol and treated
with alkaline phosphatase technique for four
hours   190X

a. Reaction of surface of epithelium
b. Reaction in capillaries under epithelium

Figure 85. Alkaline phosphatase in ampulla of oviduct -
section of oviduct area two from sow one fixed
in chilled 80 per cent alcohol and treated with
alkaline phosphatase technique for four hours
190X

a. Reaction in capillaries and connective
tissue beneath the epithelium
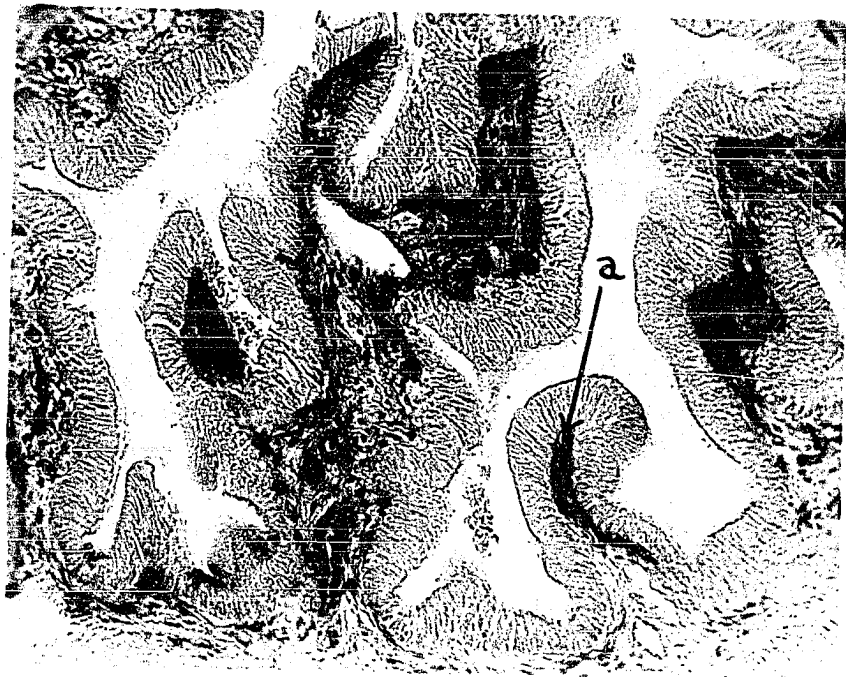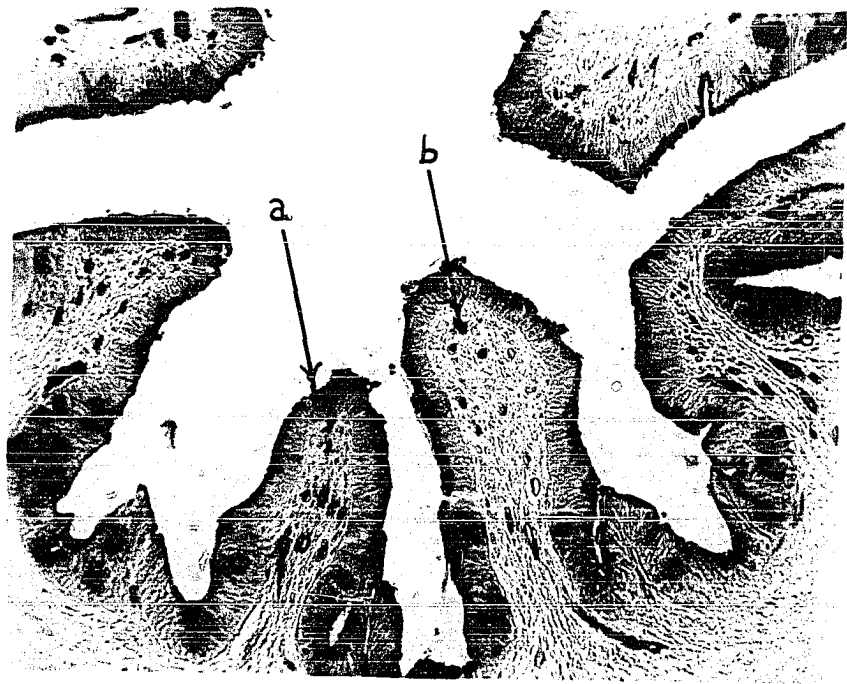
Figure 86. Glycogen in isthmus of oviduct - section of
oviduct area one from sow three fixed in
Rossman's fluid and treated with Bauer-Feulgen
technique for glycogen   495X

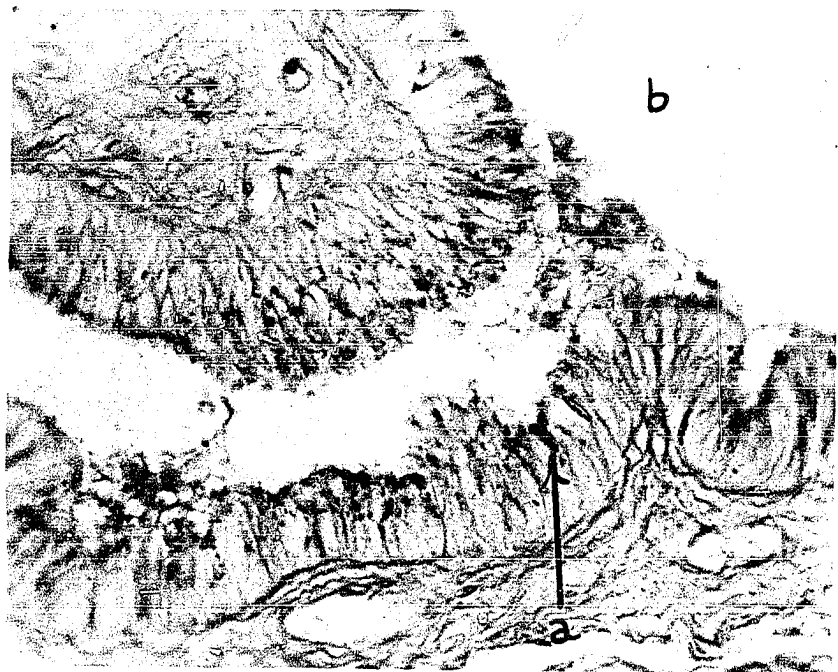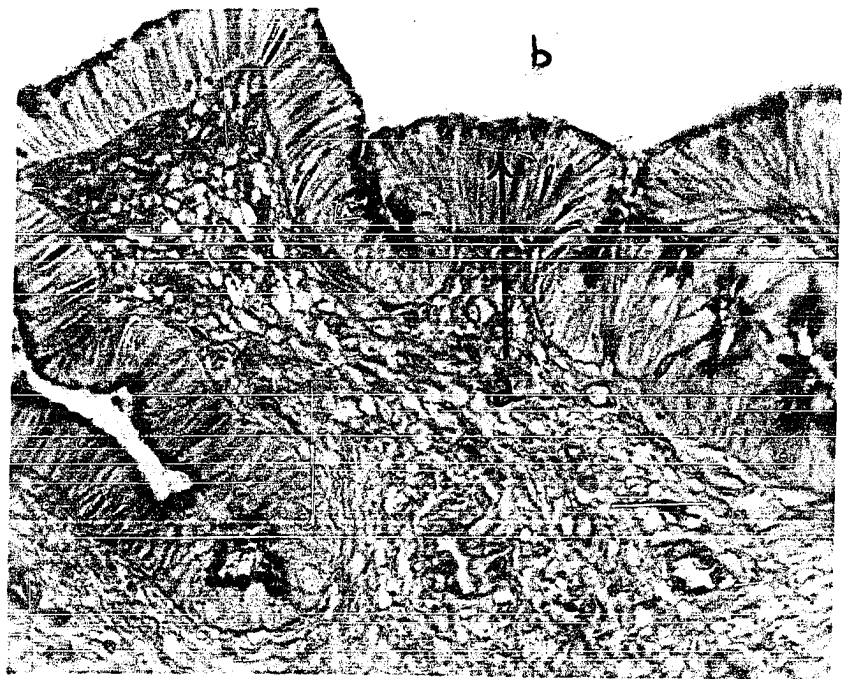a. Glycogen granules in cytoplasm of epithelial
cells near the lumen
b. Lumen of oviduct

Figure 87. Glycogen in ampulla of oviduct - section of
oviduct area two from sow two fixed in Rossman's
fluid and stained with Bauer-Feulgen technique
for glycogen   495X

a. Glycogen granules in cytoplasm of epithelial
cells
b. Lumen of oviduct

Figure 88. Lipid droplets in ovary from sow three fixed in
10 per cent formalin and stained with sudan
black B

a. Connective tissue of theca externa
b. Lipid droplets

Figure 89.   Section of uterus area three from sow four
             fixed in Zonker's formol and stained with tri-
             chrome stain   495X

             a.   Spermatozoa in lumen of uterus
             b.   Leucocytes in lumen of uterus

Figure 90.   Phagocytosis of spermatozoa - smear made from
             endometrium 105 minutes after breeding fixed in
             formal calcium and stained with Baker's hematin
             stain   895X

             a.   Phagocytized spermatozoa
             b.   Polymorphonuclear leucocytes
             c.   Spermatozoa being phagocytized
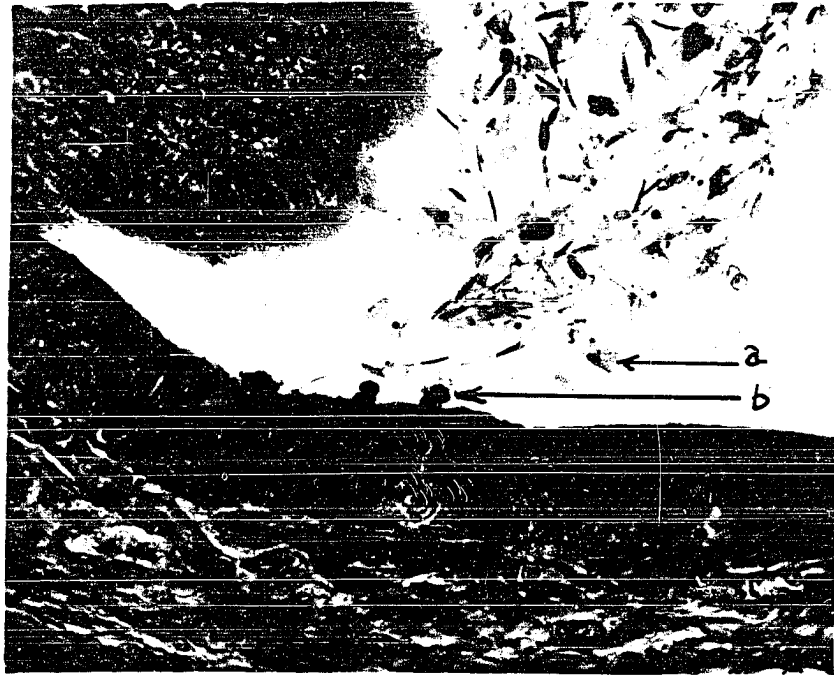
Figure 91.   Phagocytosis of sperm cells - smear made from
              endometrium of sow one 105 minutes after
              natural service by boar four fixed in 10 per
              cent formalin stained with hematoxylin and
              eosin

              a. Spermatozoa
              b. Neutrophils phagocytizing spermatozoa
              c. Sperm-cell with bent tail

Figure 92.   Phagocytosis of sperm cells - same slide as
              Figure 91

              a. Sperm-cell being phagocytized
              b. Head of sperm-cell enveloped by leucocyte
              c. Detached sperm tail
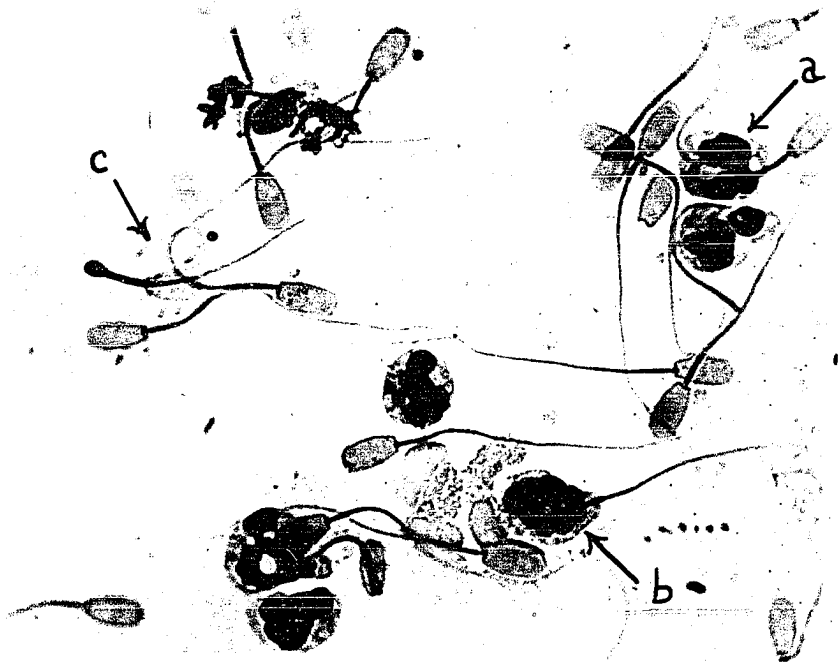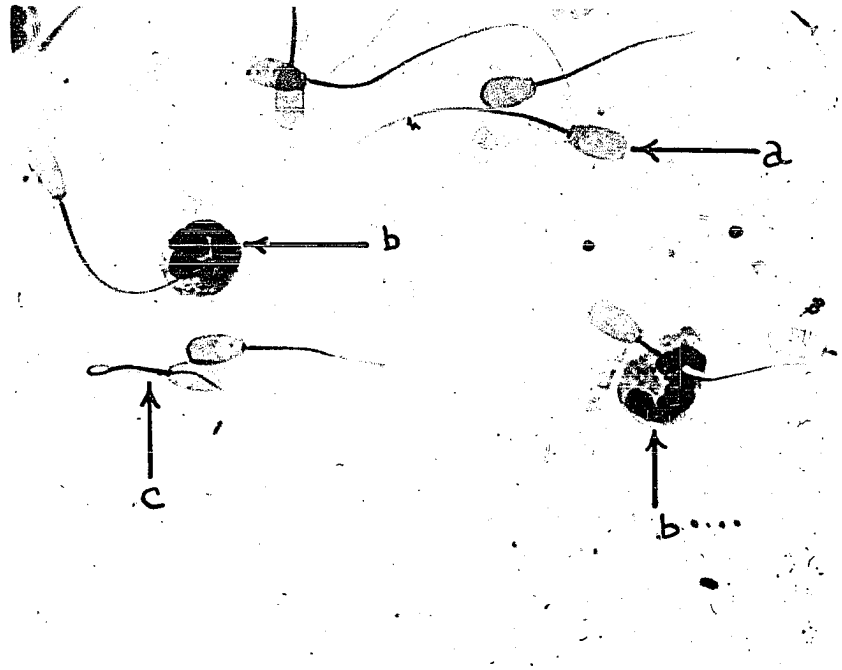
Figure 93.   Yorkshire sow # 2607 and litter of 9 pigs
              out of 16 farrowed following artificial in-
              semination with 75 cc. of raw semen 120
              minutes after collection

Figure 94.   Duroc sow # 2297 and litter of four pigs out of
              six farrowed following artificial insemination
              with 100 cc. of raw semen 70 minutes after
              collection

Figure 95.   Poland China sow # 36 and litter of 8 pigs
              remaining out of 10 farrowed following
              artificial insemination with 100 cc. of raw
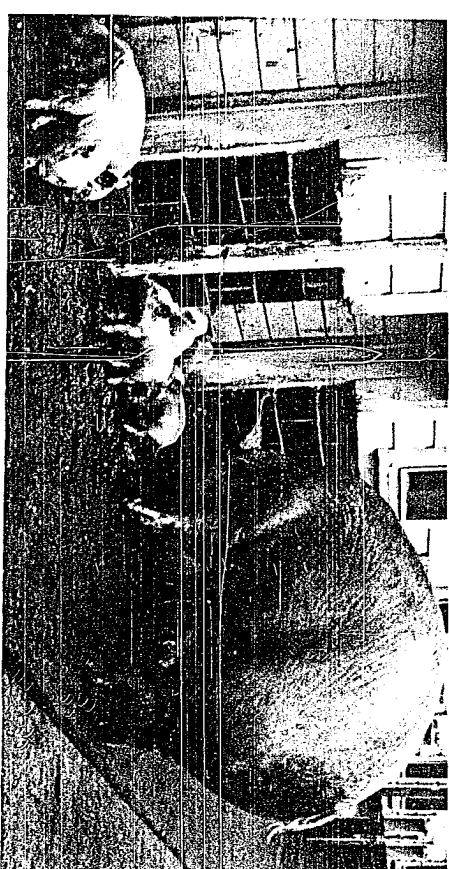   semen 125 minutes after collection

Figure 96. Lipid droplets in seminiferous tubule - section of testicle from boar six fixed in cadmium calcium formol and stained with oil red O   495X
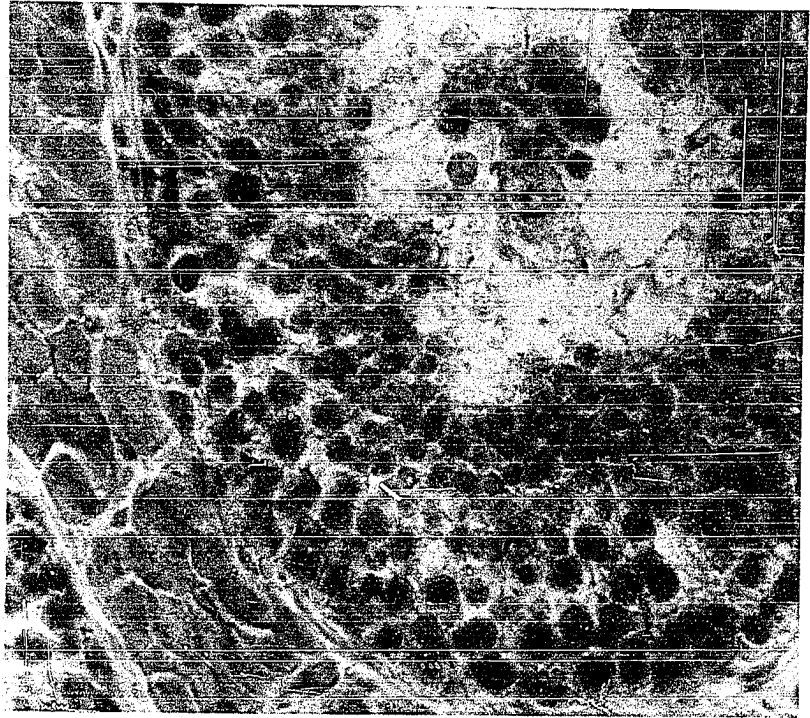
Figure 97.   Lipid droplets in epididymis - section of body
             of epididymis from boar six fixed in cadmium
             calcium formol and stained with oil red O
             190X

Figure 98.   Lipid droplets in epididymis same slide as
             Figure 97   495X
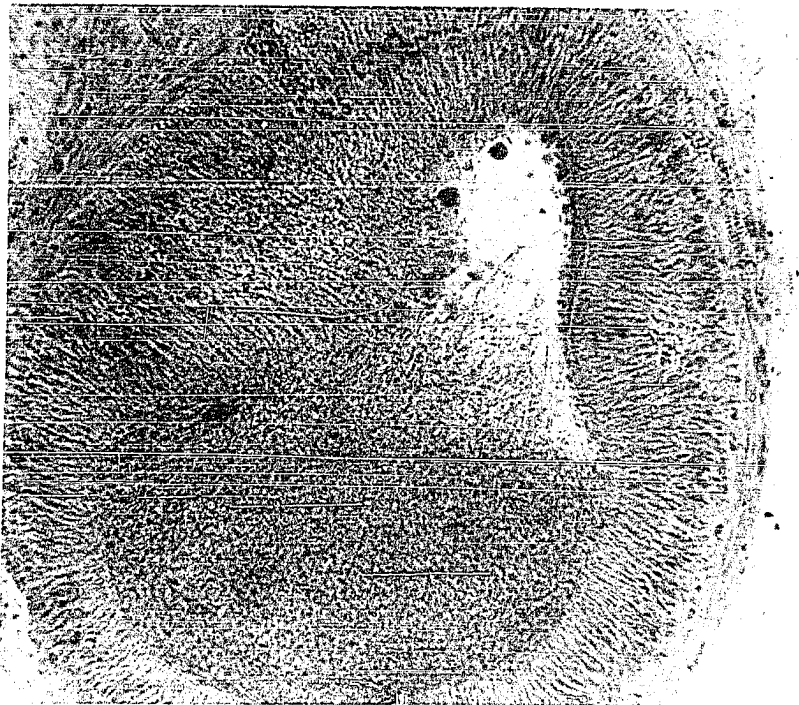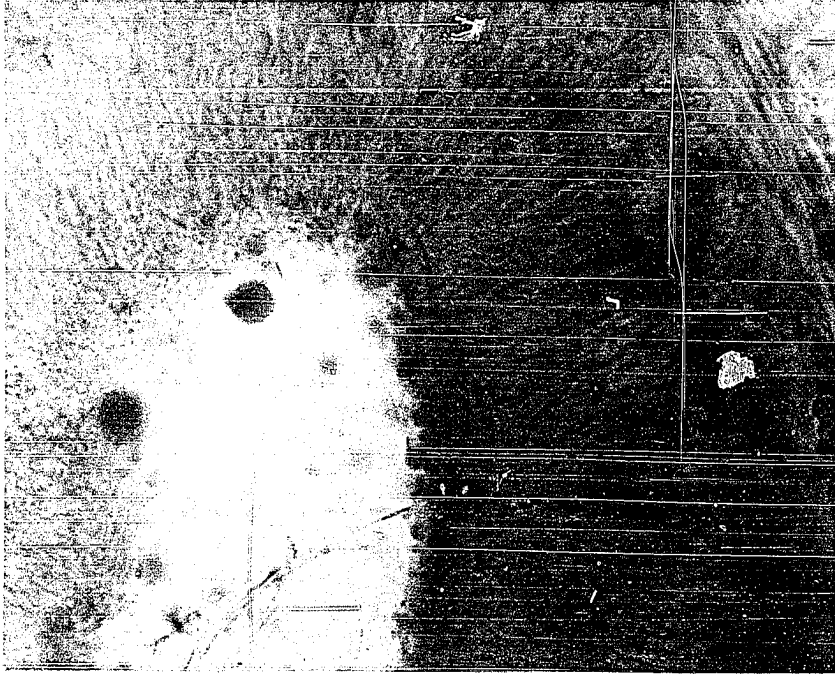
Figure 99.   Glycogen in isthmus of oviduct - section of
              oviduct area one from sow three fixed in
              Rossman's fluid and treated with Bauer-Feulgen
              technique for glycogen with picric acid
              counter-stain   495X

Figure 100. Glycogen in epididymis - section of tail of
             epididymis of boar 2 fixed in Rossman's fluid
             and stained with Bauer-Feulgen technique with
             picric acid counter-stain   495X