SUMMARY

The thesis consists of two parts. The first is a clinical and pathological description of the cystic hyperplasia-pyometra complex in the bitch; the second is a study of the experimental reproduction of the various forms of the disease.

The survey of the cystic hyperplasia-pyometra complex revealed that it is a disease of the older, nulliparous bitch which may become clinically manifest as an endometritis during the metoestral phase of the oestrus cycle. It was possible to divide the cases into four broad groups on a histological basis. A gradation of histological changes could be traced from uncomplicated cystic glandular hyperplasia of the endometrium through acute endometritis to a plasma cell endometriopathy and thence to a chronic endometritis. It was possible to correlate ovarian changes with those in the endometrium. Uncomplicated cystic glandular hyperplasia was observed at all stages of the cycle. In acute endometritis, the morphological appearance of the corpora lutea indicated that they were always active even at a stage in the cycle when they should normally have regressed. The plasma cell lesion was observed only in association with regressing corpora lutea. In the majority of the chronic cases, the corpora lutea had regressed though a few appeared active long after the normal period of regression.

It is concluded from this survey that the aetiology and pathogenesis

of the cystic hyperplasia-pyometra complex are intimately related to the functional state of the ovaries; a contention supported by a series of experiments to study the effects of ovarian hormones on the endometrium of the ovariectomised bitch.

Oestrogens in daily doses of 5 mg. - 25 mg. for periods up to 40 days failed to produce cystic glandular hyperplasia of the endometrium. Progesterone in daily doses of 10 mg. for up to 40days produced only metoestral changes but when it was used for 60 days after preliminary oestrogen stimulation a mild form of cystic glandular hyperplasia of the endometrium was produced.

Since the natural disease requires several oestrus cycles in which to develop, it appeared essential to simulate these by alternate treatment with oestrogen and progesterone. The first type of cycle used consisted of 10 daily injections of 5 mg. of stilboestrol dipropionate followed by 20 daily injections of 10 mg. of progesterone. The degree of cystic glandular hyperplasia produced after four cycles of this type was comparable to that occurring in natural cases. The histological picture became complicated by increased collagen deposition and uterine fibrosis if the number of cycles was increased.

Acute endometritis was produced by increasing the progesterone dosage from 10 mg. up to 50 mg. per day during the sixth cycle in one animal. Using cycles in which the progesterone dose was 25 mg. per day, cystic glandular hyperplasia was produced after two cycles and acute endometritis

supervened after four cycles. If progesterone was withheld there was relief of symptoms and spontaneous discharge of the uterine contents. The acute inflammatory reaction in the endometrium regressed and was replaced by a plasma cell infiltrate.

It was possible to reproduce the severe form of chronic endometritis by a combination of hormonal treatment and surgical interference with uterine drainage.

The pathogenetic and aetiological implications of these experiments are discussed in relation to the naturally occurring disease in the bitch.

THE CYSTIC HYPERPLASIA-PYOMETRA COMPLEX IN THE BITCH

by

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INDEX OF TEXT

SECTION 1

A description of

the naturally occurring cystic hyperplasia-pyometra complex

in the bitch.

INTRODUCTION

Pyometra is defined as an accumulation of pus in the uterus. In the veterinary field, the term is commonly used to describe a specific post-oestral syndrome of the adult bitch associated with a variety of clinical and pathological manifestations of genital disease. The majority of cases are characterised by cystic glandular hyperplasia of the endometrium upon which a complete range of inflammatory changes may be superimposed. The disease may be symptomless but more frequently it is manifested by vaginal discharge, abdominal distension, anorexia, thirst and vomiting. Little is known of its etiology and pathogenesis but it is commonly ascribed to some ovarian dysfunction.

Prior to 1900, only a few isolated cases of genital disease were reported in the dog. The gynaecology of the domestic carnivores was given only a passing mention in the early veterinary textbooks.

In 1905, Banham and Hobday, discussing a number of cases of metritis in the bitch, expressed surprise at the frequency with which the condition was observed in the virgin animal. They did not attach any particular significance to this finding.

In France, Bouchet (1906) verified the observations of Banham and Hobday concerning the occurrence of metritis in the maiden bitch. He considered that this was a distinct entity which was not related to puerperal endometritis. He suggested that the condition was chronic in nature but offered no explanation of its origin.

In the same year, Bargeon gave an excellent systematic description of the clinical features of the disease in which he reiterated the frequency of the disease in the maiden bitch. His observations led him to believe that vulval discharge in the bitch was generally associated with a metritis but rarely with a vaginitis or a cervitis. He noted that vulval discharge in chronic metritis was frequently irregular and he ascribed this to waves of mucin production. He suggested that the disease was of bacterial origin with infection from the vagina or at coitus with an infected male.

French (1906) recognised two types of endometritis in the virgin bitch which he believed were separate entities distinct from the post-parturient condition. He described a chronic catarrhal endometritis commonly found in animals of advanced age; discharge in such cases was usually of several weeks duration and was often of irregular occurrence. This disease was characterised by an acute exacerbation with loss of appetite, thirst and vomiting. He noted irregularity of vulval discharge and frequent retention of pus in the uterus, and ascribed it to inflammatory swelling of the cervix. The second condition was described as chronic proliferative endometritis in which the endometrium became hyperplastic and cystic. He did not observe clinical illness associated with this proliferative form though he considered it to be of inflammatory origin.

In 1908, Sewell added greatly to the existing knowledge of the

disease by observing that it was directly related to the oestrus cycle. He noted in several cases with recurrent attacks of endometritis that symptoms always appeared within a few weeks after oestrus. O'Brien (1908) also reported this association of chronic metritis in the bitch with recent oestrus and suggested that the disease was produced by suppression of menstruation. This hypothesis was based on the then current concept that pro-oestral bleeding in the bitch was analogous to menstruation in women.

Some 20 years elapsed before any major contribution was made to the knowledge of the disease. Möller-Sörensen (1929) reported the results of a bacteriological and pathological survey of 46 dogs with inflammatory lesions of the uterus. He divided his cases into broad groups, namely, pyometra and endometritis chronica catarrhalis. In the former, there was retention of pus within the uterus whereas in the latter there was free drainage of uterine fluid through the cervix. He could find little histological difference between pyometra and endometritis chronica catarrhalis and concluded that both were manifestations of the one disease process in which retention of pus was dependant on the degree of inflammatory involvement of the cervical mucosa. Bacteriological examination revealed similar organisms in both groups. B. coli was isolated from over 60% of the uteri examined and in 20% staphylococci or streptococci were present. He failed to isolate organisms from 15% of the specimens submitted to culture. These bacteriological findings have been confirmed

by several authors (Benesch and Fenner, 1930; Lesbouyries and Berthelon, 1935 and Tourissen, 1957).

Benesch and Fenner (1930) attempted to infect the uteri of experimental bitches with pus and pure cultures of bacteria isolated from cases of endometritis and pyometra. Material was injected via the cervical canal and directly through the uterine wall. In only a few animals did an endometritis appear and in these it did not resemble the natural condition. More recent attempts to produce an endometritis in the bitch by injecting pus or bacterial suspensions have met with scant success (Lesbouyries and Berthelon, 1935; Tourissen, 1957 and 1958; and Kostner, 1962).

Following their failure to reproduce the condition experimentally, Lesbouyries and Berthelon (1935) expressed the opinion that some predisposing factor was essential before bacterial infection could produce an endometritis in the dog and cat. In support of this, they advanced the results of routine treatment of clinical cases of endometritis by ovariectomy. Within three days, profuse vulval discharge appeared in those animals which had previously shown retention. Marked alleviation of symptoms was produced and complete regression of the endometritis followed within a few weeks. They emphasised that the discharge still contained bacteria during the period of recovery. In a two-year follow-up of these animals, there was no evidence of recurrence of the disease. From this they concluded that post-oestral endometritis in the domestic carnivores was a manifestation of hormonal imbalance due to ovarian dysfunction.

Hetzel (1935), using large doses of oestrone, produced a mild form of cystic hyperplasia of the endometrium in normal and ovariectomised bitches. The cystic dilation of the glands showed some resemblance to the glandular changes in the natural condition but the intense proliferative, hyperplastic features of the latter were absent. He inferred from his experiment that cystic glandular hyperplasia of the endometrium in the bitch was the result of prolonged hyperoestrinism and suggested that the oestrogen was derived from "persistent" and cystic follicles. He had observed the frequency with which post-oestral endometritis is associated with cystic hyperplasia and deduced that the inflammatory reaction and the occlusion of the cervix were merely further manifestations of hyperoestrinism. Despite the observations of De vita (1939) and Kostner (1942) that pyometra is commonly associated with the presence of corpora lutea in the ovaries, this conception of the aetiology of the cystic hyperplasia-pyometra complex has remained dominant since Hetzel's (1935) report.

Later workers (De vita 1952 and Teunissen 1952) have repeated Hetzel's (1935) studies on the effect of oestrogens on the canine endometrium. Both obtained mild hyperplasia of the endometrium with some degree of cystic dilation of glands but they failed to replicate the natural condition.

Teunissen (1952), following his failure to reproduce the disease using oestradiol monobenzoate, treated a number of ovariectomised bitches

with a wide range of doses of progesterone. He obtained some glandular proliferation in 15 animals and in seven of them, an acute inflammatory reaction occurred. The glandular proliferation obtained in these experiments with progesterone approximated more closely to the natural condition than the effects produced by oestrogens. Despite the lead given by the work of Teunissen, no report of the production of an endometritis exhibiting the clinical and pathological characteristics of the cystic hyperplasia-pyometra complex has been published.

The work presented in this thesis was undertaken to provide a detailed clinical and pathological description of the disease in its various forms. No complete study of both clinical and sub-clinical forms of the disease has been published. From such a study, it was hoped to obtain some information which would help to elucidate the pathogenesis and aetiology of the condition. Experimental work was started to reproduce cystic hyperplasia of the endometrium of the dog. By utilising the information obtained from the survey of the natural condition, it was hoped that the various inflammatory changes might be superimposed on experimentally produced cystic hyperplasia.

Using ovarian hormones, it has been possible to reproduce all the forms of the cystic hyperplasia-pyometra complex observed in the survey of the natural disease.

THE OESTRUS CYCLE OF THE BITCH

INTRODUCTION

A study of the normal cyclic processes in the genital organs of the bitch was essential as a base line for reference in the analysis of natural and experimental changes. Evans and Cole (1931) gave an excellent description of the various stages of the canine oestrus cycle. They found that the average bitch experiences two periods of oestrus or heat per year at approximately six-monthly intervals. Following the suggestions of Heape (1900), they proposed a number of logical stages for division of the canine oestrus cycle:

1) Pro-oestrus is the period from the first appearance of sanguinous discharge from the vulva to the time of first acceptance of the male. During this stage which usually lasts for nine or ten days, the bitch is attractive to males but will not permit coitus.

2) Oestrus is the period during which the bitch will submit to copulation. Oestrus lasts approximately nine or ten days and is characterised by diminution of discharge and marked vulval oedema.

3) Metoestrus is the period of development and regression of the corpora lutea. It lasts for 90 days in the non-pregnant animal.

4) Anoestrus is the stage of sexual quiescence extending from the end of metoestrus to the start of the next pro-oestrus. This interval is usually eight to ten weeks.

This subdivision of the oestrus cycle will be used in the subsequent

description of a study of the normal genital organs. The latter corroborates the work of Evans and Cole (1931) and Mulligan (1942) and in addition describes some histochemical reactions of the endometrial mucin.

Material Studied

The description is based on the study of the genitalia of 25 bitches which were ovaro-hysterectomised at various known stages in the oestrus cycle. In addition, organs were obtained at autopsy from 185 bitches of which details of breeding and oestral history were available.

Histological Examination

Ovary

The ovary of the anoestrus bitch reveals a thin layer of primordial follicles situated almost directly under the germinal epithelium. In the inner cortex, there is a narrow zone of small follicles some of which are developing whilst others are degenerating. In animals which have undergone a previous cycle, small remnants of corpora lutea are commonly present. The ovarian stroma is dense and highly cellular at this stage. The stromal cells are spindle-shaped with only a moderate amount of cytoplasm and a comparatively large, finely reticular, ovoid nucleus.

Conspicuous changes occur in the cortex during pro-oestrus. There is considerable increase in the number of small and medium follicles. At this stage it is possible to discern those follicles which are destined to ovulate. The amount of liquor folliculi increases. There is marked proliferation of the granulosa cell layer which is thrown into elaborate

folds which are invaginated with vascularised cones of fine connective tissue from the theca interna. While these follicles enlarge, the number of degenerating follicles increases.

At about the second day of oestrus ovulation occurs and following this the granulosa cells increase in size until about the 10th day. The cells are large and ovoid with a pale eosinophilic cytoplasm stippled with tiny vacuoles. The nucleus at about the 10th day after ovulation occupies one fifth of the cell and is large and vesicular with a prominent nucleolus. The central cavity of the corpus luteum then rapidly fills with granulosa lutein cells and fine, vascular connective tissue. The obliteration of the cavity is usually complete by the 20th day after ovulation.

In the non-pregnant bitch, the first signs of regression appear in the corpus luteum just before the 30th day of metoestrus. There is a gradual increase in the amount of connective tissue and shrinkage in the total volume of the corpus luteum. By the end of metoestrus, the corpus luteum is broken up by connective tissue trabeculae and is composed of small cells in which the cytoplasm is scanty or is completely vacuolated.

Uterus

During anoestrus, the muscle layers, comprising inner circular and outer longitudinal, are relatively inconspicuous. The endometrium may be divided into three zones: (a) the basalis zone in which small polyhedral glands ramify; (b) the tubular zone in which tubules run from the basal glands to the endometrial surface; and (c) the crypt zone

which is produced by indentations of the superficial epithelium and by glandular orifices. At this stage, the epithelium of all layers is simple low columnar or cuboidal in type (Fig. 1).

With the onset of pro-oestrus, the endometrium becomes markedly thickened. The stroma becomes oedematous and the tubular zone is disproportionately increased in width (Fig. 2). All blood vessels are dilated and the capillaries of the crypt zone are congested. Extravasation of red blood cells occurs in the immediately subluminal stroma. This haemorrhage may be focal or it may form a complete zone around the superficial and crypt epithelium. No break in continuity of the superficial epithelium can be found to account for the blood stained discharge. The epithelium of all layers is a tall columnar type. These changes progress through oestrus into the second week of metoestrus. The basal zone increases in thickness due to branching and hypertrophy of the glands (Fig. 3). The gland epithelium is increased in height, mitotic figures are present and pseudostratification is distinct. The tubular epithelium exhibits similar changes. The crypts are more numerous and are dilated. Retrogressive changes begin in the 4th week after oestrus and continue throughout the remainder of metoestrus. The glands become rudimentary and by the 70th day, the endometrium resembles that of anoestrus except for clear, stout columnar, fat-containing cells covering the surface. This superficial epithelium is later sloughed and is replaced by new epithelium regenerated from the crypts.

The Histochemistry of Endometrial Mucin

Small supranuclear vacuoles are present in the glandular and crypt epithelium during oestrus and the first 14 days of metoestrus. These vacuoles have generally disappeared by the 3rd week of metoestrus when mucin becomes evident in the lumina of the glands and crypts. During anoestrus, occasional lumina still contain a small amount of mucin. From the staining reactions of both intracellular and luminal mucus, it is evident that it contains acid mucopolysaccharide.

Neutral fat is present only during late metoestrus when it is found in the superficial epithelium.

Table I

Details of staining reactions of endometrial mucin.

Stain	Anoestrus	Pro-oestrus	Oestrus	Early Metoestrus	Late Metoestrus
P.A.S.	No secretion	No secretion	+	+	-
Diastase +P.A.S.	"	"	+	+	-
Toluidin Blue	"	"	Metachromasia	Meto-chromasia	-
Methylene Blue Extinction	"	"	pH2	pH2	-
Southgate's Mucicarmin	"	"	+	+	-
Alcian Blue	"	"	+	+	-
picro-Mallory	"	"	+	+	-
Sudan Black B	"	"	-	-	+

Vagina

Throughout anoestrus, the vaginal epithelium is low and consists of only two layers of cells. The basal layer is composed of cuboidal cells with scanty cytoplasm. The nuclei are dense and ovoid with their long axes orientated at right angles to the basement membrane. The superficial layer is composed of irregularly columnar cells with more abundant cytoplasm and pale round nuclei.

With the onset of pro-oestrus, there is active proliferation of the vaginal epithelium so that by the first day of oestrus it may be composed of from 12 to 20 layers of cells. The basal cells increase in size and in number; the nuclei become pale and rounded and mitotic figures are frequent. The superficial 8-10 layers form a well defined stratum corneum. As oestrus progresses there is a reduction in the number of cell layers due to surface desquamation and by the first day of metoestrus only 4 to 6 layers remain. During the first 10 days of metoestrus there is a striking infiltration of neutrophils into the lamina propria and epithelium; the invading cells rapidly disappear over the next 5-8 days. By the 20th day of metoestrus, the superficial epithelium has become reduced to two layers of cells in which state it persists till the onset of the next pro-oestrus.

Determination of the Stage of Cycle by Examination of Vaginal Smears

During anoestrus, cells are not numerous in the vaginal smear and only two types of epithelial cells are observed:

(a) large cells, ranging from 30-50 microns in diameter; their pale eosinophilic cytoplasm is often vacuolated or granular. These cells may be round, elliptical or almost square. The nuclei are large and pale with a prominent nucleolus.

(b) small cells with scanty, basophilic cytoplasm. These cells are rounded or almost fusiform in shape and they possess small, dense hyperchromatic nuclei. They frequently appear in small sheets of 6-10 cells.

In contrast to the picture observed in rodents, leucocytes are never numerous in vaginal smears from the bitch in anoestrus. Daily fluctuations in leucocyte counts occur and many smears contain none. Erythrocytes are never present during anoestrus.

The onset of pro-oestrus is heralded by a number of changes in the cell picture of the vaginal smear. Initially, some of the large eosinophil epithelial cells become more angular in shape and the nuclei become pyknotic and hyperchromatic. As pro-oestrus progresses, the cells become more and more cornified until on the day of first acceptance of coitus, all the epithelial cells are cornified. The cornified cells of the bitch, unlike those of rodents, are almost always nucleated, though the nuclei are usually pyknotic. In the first few days of pro-oestrus, a moderate number of large epithelial cells with basophilic cytoplasm are seen but these soon disappear. The small basophil epithelial cells persist until the onset of oestrus but then disappear until the beginning of metoestrus. The most striking feature of pro-oestrus is the appearance of great numbers of erythrocytes which reach

their peak concentration towards the end of this stage of the cycle. The erythrocytes decrease in number throughout oestrus and are absent by the 10th day of metoestrus.

In the last few days of oestrus neutrophil leucocytes appear in the smear; they increase in number over the first few days of metoestrus but disappear by the 15th day of metoestrus and are only occasionally observed until anoestrus.

Cornified epithelial cells persist in progressively decreasing numbers until the 15th day of metoestrus and are not observed again until the following pro-oestrus. Metoestrus smears show a gradual return to the anoestrus state though numbers of large basophil epithelial cells are observed until the end of this stage.

Full details of the differential vaginal cell counts at various stages of the oestrus cycle in 25 normal bitches are given in Table 2.

Table 2

Differential cell count of vaginal smears in the bitch

	Epithelial Cells				Blood Cells	
Stage	Cornified	Eosinophil	Large Basophil	Small Basophil	Neutrophils	Erythrocytes
Anoestrus	0	69% (64-76)	0	31% (24-36)	27 (0-240)	0
Early Pro-oestrus	5% (1-10)	18% (8-30)	37% (28-45)	40% (32-50)	4 (0-10)	230 (50-410)
Late Pro-oestrus	39% (30-52)	36% (27-51)	11% (4-17)	14% (7-18)	16 (3-37)	1970 (800-3100)
Early Oestrus	73% (61-90)	27% (10-39)	0	0	21 (9-35)	420 (290-630)
Late Oestrus	96% (93-100)	4% (0-7)	0	0	60 (39-75)	120 (97-139)
10th day Metoestrus	27% (21-37)	41% (36-47)	0	32% (16-43)	319 (197-576)	0
60th day Metoestrus	0	38% (27-46)	20% (17-31)	42% (35-47)	0	0

Materials and Methods

The 100 cases which form the basis of the description of the natural disease were all animals presented for treatment at the University of Glasgow Veterinary Hospital during the years 1954-1957. Of these, 79 were tentatively diagnosed as suffering from some form of the cystic hyperplasia pyometra complex on the evidence of the owner's account of the history of the case and of a physical examination. The other 21 cases were discovered during a survey of the diseases of the canine genitalia carried out in the course of routine post-mortem examinations made in the Hospital Pathology Unit.

In the early part of the investigation, laboratory and other ancillary examinations were carried out with the primary aim of establishing the diagnosis and of assessing the severity of the disease.

As a routine procedure, all suspected cases were submitted to radiographic examination. Exposures of the abdomen were taken in lateral and in dorsal recumbency. Increased contrast was obtained by artificial pneumo-peritoneum in selected cases.

Vaginal smears were taken from every animal. The vagina was cleaned by douching with normal physiological saline and dried with a tampon of cotton wool. The vulval lips were parted using a small speculum and a metal spatula with a curved end was inserted to a depth of 5-7 cms. i.e. into the upper vagina. The curved point of the spatula was pressed against the vagina wall to obtain a thin superficial scraping. The

tip of the spatula was applied to a drop of saline on a clean slide to obtain a thin smear which was then dried and stained by Giemsa. A differential count was made of 100 epithelial cells and, at the same time, the number of leucocytes and erythrocytes observed was recorded.

Haematological examinations performed included calculation of erythrocyte and leucocyte counts per c.mm. and estimation of haemoglobin as oxyhaemoglobin by the method of Bell, Chambers and Waddell (1945). The erythrocyte sedimentation rate (E.S.R.) and packed cell volume (P.C.V.) were measured. Freshly prepared blood films were stained by Leishman's method and a differential leucocyte count was made by examining 200 cells.

In all cases, serum urea concentration was estimated by the Urease and Nesslerisation method described by Harrison (1947) and serum bilirubin was estimated by the method of Malloy and Evelyn (1937). In certain cases, the following estimations were made:-

1. Serum calcium by the method of Kramer and Tisdall (1921);
2. serum inorganic phosphate by the method of Fiske and Subbarow (1925);
3. serum alkaline phosphatase by the method of King, Abul-Fadl and Walker (1951);
4. serum proteins by the Biuret method;
5. the albumin/globulin ratio by paper electrophoresis.

The routine procedure adopted in the hospital in treatment of the condition was complete ovaro-hysterectomy. In a few critically ill animals, marsupialisation was performed initially and when the animal's

condition had improved sufficiently, ovaro-hysterectomy was carried out.

The uterus and ovaries were examined as soon as possible after surgical excision. The number of corpora lutea and other recognisable structures in each ovary was noted. The weight, dimensions and shape of the uterus were recorded. The degree of patency of the cervical canal was measured and a sample of the uterine fluid was obtained using an aseptic technique.

Blocks of tissue for histological examination were taken from both ovaries, from the upper, middle and lower thirds of both uterine horns, from the corpus uteri and from the cervix. These tissues were fixed in formol-sublimate, dehydrated and cleared in alcohol-amyl acetate-benzol series and double-embedded in celloidin and paraffin. Routine staining was by haemalum and eosin; other methods used on selected sections from each case were picro-Mallory, Gordon and Sweets's reticulin stain, toluidine blue, alcian blue, Best's carmine, Southgate's mucicarmine, Sudan Black B, periodic acid-Schiff before and after hydrolysis with diastase, methylene blue in buffers of a range of pH values, pyronin-methyl green and Wiegert's elastin stain. In some cases, frozen sections were stained by Sudan IV.

Classification

In the following description of the natural disease, each case has been assigned to one of four groups; the criteria adopted in classification are morphological being based on the histopathological appearances

of the uterus. A high degree of correlation was found between the histological changes in the genital tract and the clinical, haematological and biochemical findings. It is considered that this pathological classification is a useful one for a general description of the disease complex.

The four groups are:-

1. Cystic glandular hyperplasia of the endometrium.
2. Cystic glandular hyperplasia and plasma cell infiltration of the endometrium.
3. Cystic glandular hyperplasia with acute endometritis.
4. Chronic endometritis.

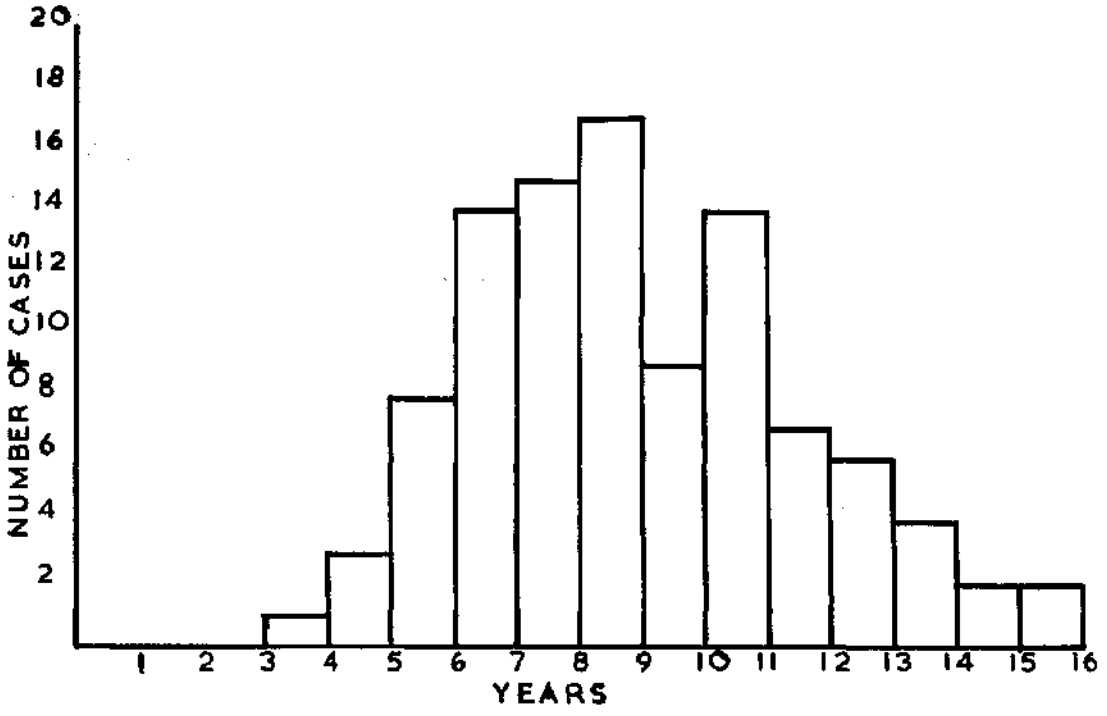
The Incidence of the Cystic Hyperplasia-Pyometra Complex in relation to age, parity and breed of dog affected.

From Fig. 4, it is apparent that the incidence of the disease is highest in middle age with peaks at eight and 10 years. The mean age is 8.2 ± 2.4 years with a range of 3 - 15 years. Only 4% of the animals examined were under 5 years old. The gradual decrease in numbers after 10 years does not necessarily indicate a decreased incidence of the disease, because the average life span of the dog is probably less than 10 years. In the present series, 19% of the animals were over 10 years of age but it is noteworthy that of 500 consecutive

dogs admitted to the hospital, only 9% were in this age group. Full details of the age incidence of bitches in the various groups are given in the appropriate sections.

Fig. 4.

Age Incidence of affected Animals



Parity. Breeding records were available in 94 cases. Of these 73 were virgins and only 6 had whelped more than one litter. The incidence of nulliparous bitches was of the order of 75% in each of the

groups, (Table 3).

Table 3

The Incidence of Parity in affected Animals

Group	Virgin	One Litter	Two Litters	Three or more Litters
I	15	6	1	0
II	13	4	0	0
III	38	5	2	1
IV	7	0	2	0
Total	73	15	5	1

Breed Incidence

The numbers of the different breeds in all groups are given in Table 4.

Table 4

Distribution of Breed Incidence

Breed	Group I	Group II	Group III	Group IV	Total
Mongrel Terrier	4	2	10	3	19
Collie	5	2	6	1	14
Cairn Terrier	0	2	2	2	6
Cocker Spaniel	2	0	3	0	5
Bull Terrier	1	0	3	1	5
Scottish Terrier	1	2	1	1	5
Black Labrador	2	0	2	0	4
Golden Labrador	0	1	1	2	4
Mongrel Spaniel	2	1	1	0	4
Alsatian	0	1	2	0	3
Smooth Haired Fox Terrier	0	2	1	0	3
Wire Haired Fox terrier	1	0	2	0	3
Springer Spaniel	1	1	1	0	3
Boxer	1	1	0	0	2
Dachshund	2	0	0	0	2
Welsh Corgi	0	0	2	0	2
Pekingese	0	0	2	0	2
Poodle	0	0	2	0	2

Table 4 (continued)

Distribution of Breed Incidence

Breed	Group I	Group II	Group III	Group IV	Total
Airedale	1	0	1	0	2
Great Dane	0	0	1	1	2
West Highland Terrier	0	0	2	0	2
Bull Mastiff	0	0	1	0	1
Pomeranian	0	1	0	0	1
Schiperke	0	0	1	0	1
Irish Terrier	0	1	0	0	1
Irish Setter	0	0	1	0	1
Scalyham	0	0	1	0	1
Total	23	17	49	11	100

GROUP 1

This consists of 23 cases in which the basic lesion of cystic glandular hyperplasia of the endometrium is present without any superimposed inflammatory reaction.

Age Incidence.

The distribution of age incidence of affected animals is shown in Fig. 5. The bitches ranged from 3 to 12 years with a mean of 7.1 ± 2.4

years. Only 3 cases were under 5 years of age.

Breeding History

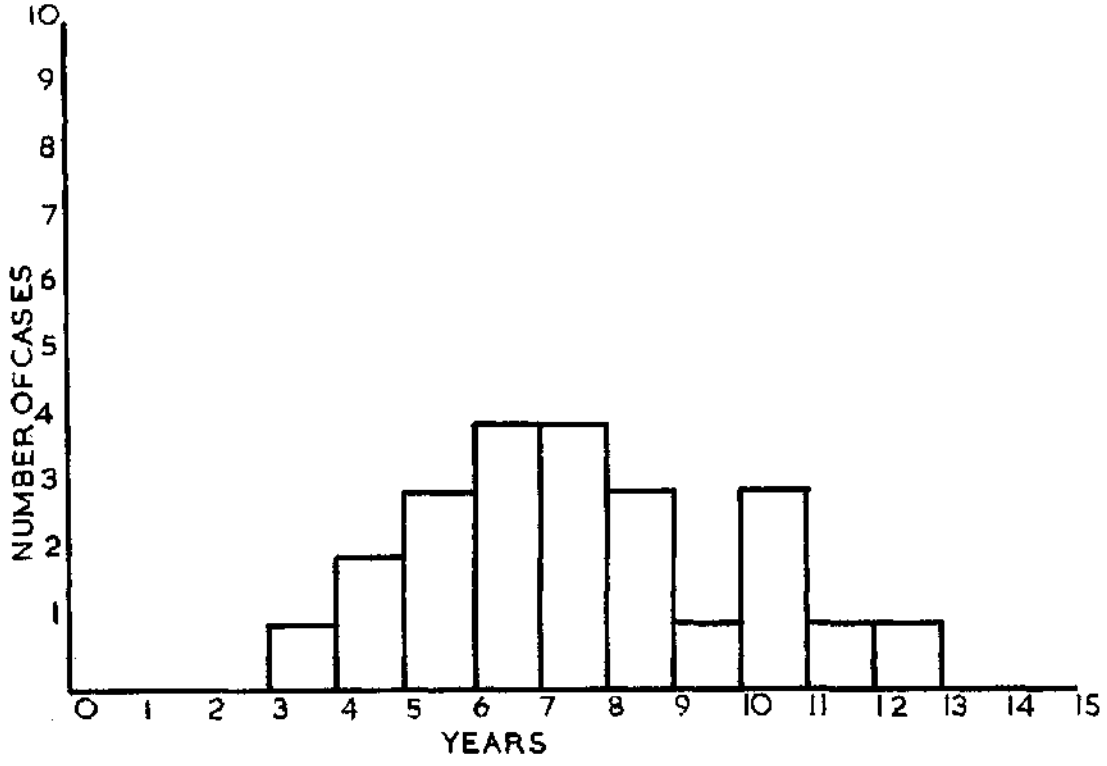
Sixteen bitches were nulliparous. Of the parous animals, six had whelped only once and one had borne two litters. The interval between parturition and examination ranged from 3 to 8 years. Six bitches had shown evidence of phantom pregnancy with all the signs of imminent parturition at 9 weeks after oestrus. Two of these were admitted to the hospital as possible cases of dystocia but thorough examination ruled out the possibility of pregnancy. The uteri of these animals exhibited typical cystic glandular hyperplasia. The owners of 9 of the maiden bitches were certain that their animals had never been mated since they were always exercised on a leash. Three were known to have been mated in recent years without establishment of pregnancy. The mating history was uncertain in the remaining four cases.

Oestral History

Bitches were observed with cystic glandular hyperplasia of the endometrium at all stages of the oestrus cycle. It was ascertained that 15 of these animals had exhibited some evidence of sexual abnormality in the years immediately prior to examination. In five cases there had been irregularity in the frequency of occurrence of oestrus for as long as 4 years before admission. The interval between any two consecutive oestrus periods ranged from three to ten months but in no instance was

Fig. 5

The age incidence of cases of uncomplicated cystic glandular hyperplasia of the endometrium.



this interval consistent over a number of cycles in any one animal. In four bitches, there had been abnormal duration of pro-oestrus and oestrus which were extended to 30 days or more, or were abbreviated to as little as five days. In some cases, the haemorrhagic vulval discharge characteristic of pro-oestrus persisted throughout oestrus and in others, the only evidence of sexual activity at oestrus was slight vulval oedema

and attraction for males. It seems possible that in some cases, oestrus signs may have been so slight that they escaped the notice of the owners.

Clinical Signs

With the exception of four cases, these animals were submitted by their owners for examination and treatment for other diseases. Physical examination revealed no general upset referable to genital disease. In the 11 cases which were in metoestrus, a slight mucoid discharge was not sufficiently profuse to cause the owners to present them for treatment. As stated above, two cases were admitted because of phantom pregnancy.

Radiography

In the course of routine examination, plates of the abdomen were taken in 14 cases and in five of these, the uterus was recognisably thickened. Radiographs taken using the pneumo-peritoneum technique showed distinct hypertrophy of the uterus in the four cases presented for treatment of genital disease.

Haematology

Oxalated samples of blood from 21 cases were examined and no abnormality was observed.

Biochemistry

Plasma urea and serum bilirubin levels were estimated in 17 cases.

No relevant change was found. Serum calcium, inorganic phosphate, protein and albumin/globulin ratios were calculated in 7 cases. No abnormality was detected.

Vaginal Cytology

Vaginal smears were obtained from 17 cases. Differential cell counts were characteristic of the stage of cycle except in a few animals believed to be in anoestrus. These showed differential cell counts suggestive of early pro-oestrus and it is probable that these bitches were prematurely entering pro-oestrus.

Morbid Anatomy

The uteri varied in size with the degree of cystic glandular hyperplasia present but few exceeded two cm. in diameter. The majority were only slightly thicker and more definitely rounded than normal. Increase in length was common and the restraining influence of the broad ligament of the uterus tended to coil the horns. In a few metoestrus specimens, the uterine lumen contained a small amount of clear, mucoid fluid. The uterine wall was thickened, largely due to endometrial hypertrophy. The endometrium was lined by clear, translucent cysts of up to one, and occasionally, two cms. in diameter (Fig. 6). In general, the cysts were scattered evenly throughout the length of the uterine horns but in less than 25% of the number they were confined to the upper ends of the horns. The corpus uteri was always less severely affected. Where hyperplasia was marked, the endometrium was thrown into folds and often

formed villous and polypoid projections into the uterine lumen. Large single polypi were not observed. In every case, the condition was bilateral and appeared to affect both horns to the same extent.

Bacteriological Examination

Using an aseptic technique, a swab was taken from the uterine lumen in every case. B. coli was isolated from six specimens which were in the early metoestrus stage. Bacteria were not obtained at any other stage of the cycle.

Histological Examination

The histological picture varies to a certain extent with the stage of cycle but an absolute increase in the number of glandular elements in all strata of the endometrium is constantly found. Whereas in any phase of the normal oestrus cycle there is a striking uniformity in the size and configuration of the glands (Fig. 3), in cystic glandular hyperplasia, there is marked disparity. Some are large and cystic whilst others in the immediate vicinity are small and apparently normal (Fig. 7). The normal histological division of the endometrium into superficial or crypt zone, tubular zone and basalis is lost. In some cases, the larger cysts appear to arise in the superficial endometrium and project into the uterine lumen; in other cases, they appear to arise deep in the basalis zone and dilate to span the entire width of the endometrium (Fig. 8). The larger cysts are generally round on cross-section but the smaller

ones may be round, star-shaped or even branched. In some cases, it is possible to differentiate two distinct layers of cysts; a basal layer of closely packed smaller cysts and a superficial layer of large cysts separated by an almost gland-free zone (Fig. 9). Papillary projections into the lumina of cysts are not uncommon (Fig.10). No definite correlation between the size of the cysts and the stage of cycle can be found though large cysts seem to be more numerous in mid-metoestrus.

The superficial epithelium during anoestrus and pro-oestrus is similar to that seen in the normal endometrium but as metoestrus begins, the increase in height and the crowding of the cells is more marked. The gradual regression of the superficial epithelium which is normally seen after the fourth week of metoestrus is not apparent in many cases of cystic glandular hyperplasia of the endometrium. The cells remain hypertrophied and pseudo-stratification is prominent with small foci of epithelial proliferation forming tuft-like projections into the uterine lumen (Fig.11). At this stage, which may be from 30 - 50 days after oestrus, the superficial and crypt epithelia become indistinguishable. Crypt formation during oestrus is often more pronounced than normal and in uteri examined after the first 20 - 30 days post-oestrus, they are widely dilated so that the endometrial edge has a scalloped outline. In many cases examined in the period 30 - 50 days after oestrus, the superficial and crypt epithelium has assumed a very florid, highly secretory appearance. The cells are very tall and the nuclei are often

nearer the luminal edge than the basement membrane. The cytoplasm is palely eosinophilic, finely vacuolated and granular. In some cells the cytoplasm is extruded from the surface in the form of small papillae which occasionally contain nuclei. In late metoestrus, the cytoplasm of the superficial epithelium becomes almost completely vacuolated and fatty in appearance. The nuclei are hyperchromatic and pyknotic and are situated close to the luminal edge of the cells. This change is seen in the endometrium of normal animals in late metoestrus but it does not extend to the crypt epithelium as it does in those showing cystic hyperplasia.

The type of epithelium lining the cysts can not always be correlated with the degree of distension of the cysts nor is it always compatible with the stage of cycle. The morphology of the cyst epithelium varies throughout any one section and may even vary in different levels of the one cyst. Flattened non-secretory epithelium is seen in many cysts, particularly in the smaller ones even during the early metoestrus phase when secretion is normally at its height. The nuclei of these epithelial cells are ovoid and are orientated with their long axes parallel to the lumen of the cyst (Fig.12). In many such cysts, secretion may not be observed on paraffin sections whilst in others, the cyst is filled with a pale eosinophilic fluid. Neighbouring cysts may exhibit a tall secretory epithelium such as is seen in the normal metoestrus glands. The large ovoid nuclei are basal in position and are orientated at right

angles to the lumen. The cytoplasm is brightly eosinophilic and contains fine supranuclear vacuoles. In such cysts, the epithelium appears to be actively secretory. In some cysts, an unusual degree of proliferation and hypertrophy of the lining epithelium may be seen with pseudostratification similar to that seen in the superficial epithelium. Cysts of this type are usually superficially placed and form polypoid projections into the uterine lumen. Occasionally, these polypoid cysts undergo necrosis, presumably due to torsion of the slender pedicle. In cysts lined by this highly proliferative epithelium, the secretion is closely adherent to the surface of the cells with streams of mucin appearing to flow from individual cells. It is not uncommon to find cysts in which the epithelium nearest the uterine lumen is tall and highly secretory but gradually decreases in height to become cuboidal in the base of the cyst.

Stromal proliferation is rarely pronounced; in most cases cystic glandular hyperplasia predominates and may almost completely fill the endometrium. In the majority of cases, stromal changes are merely an exaggeration of normal cyclic phenomena. The mild haemorrhage of the crypt zone, which is characteristic of normal pro-oestrus, is commonly more intense. Focal haemorrhages are sometimes observed in the stroma of the basalis zone and, rarely, into the lumina of cysts. Haemosiderin-containing macrophages are numerous in the endometrium of cystic hyperplasia cases during metoestrus. Oedema of the basalis zone is always

prominent in normal oestrus but it persists far into metoestrus in many cases of hyperplasia. The superficial capillaries are commonly dilated and, in some cases, moderately large vessels are present in the superficial stroma. In the normal endometrium, large vessels are seen only in the basalis zone close to the myometrium and are prominent only during oestrus. Mitotic figures are rare among the stromal cells. An impression of increased stromal cellularity may be obtained where cystic dilation causes compression of the stroma. The reticulin fibres round the cysts are always intact but are compressed. The collagen fibres appear normal in number and in structure though the periglandular arrangement is more clearly defined because of the cystic dilatation.

There is no evidence of inflammatory cells in the stroma of any of the cases in this group.

The myometrium is rarely hypertrophied but dilatation of the vessels of the stratum vasculare is commonly seen in cases which are in metoestrus. In seven cases there is adenomyotic extension of endometrial glands into the myometrium. The aberrant endometrial tissue extends through the inner layer of muscle as far as the stratum vasculare but in no case can it be seen in the outer myometrium. The adenomyotic tissue exhibits the same changes as the endometrium and in four instances shows considerable cystic dilatation (Fig.14). Only in three cases is there recognisable endometrial stroma in association with the glands. Hyperplasia of the surrounding myometrium is present in

association with most adenomyotic foci.

GROUP II

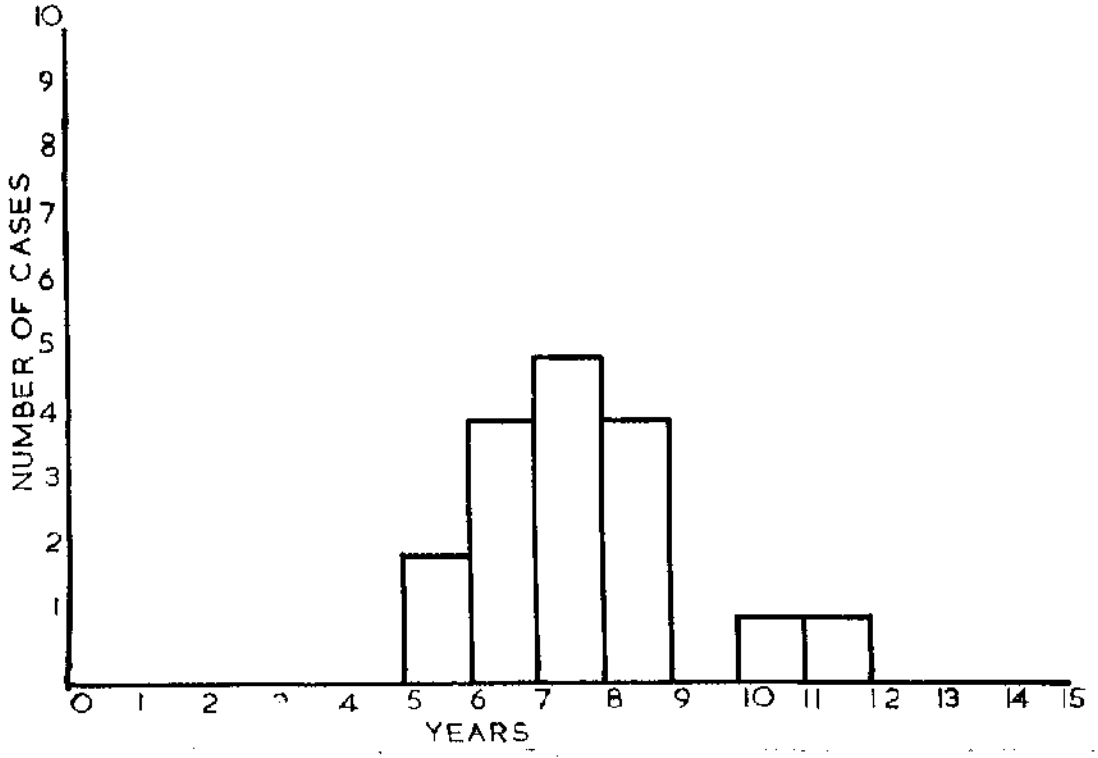
This group differs from the previous one in that there is a plasma cell infiltration superimposed on cystic glandular hyperplasia of the endometrium; it consists of 17 cases.

Age Incidence

The distribution of age incidence of affected animals is shown in Fig.15. They ranged from 5 to 11 years with a mean of 7.2 ± 1.9 years.

Fig.15.

Age incidence of cases of cystic glandular hyperplasia associated with plasma cell infiltration.



Breeding History.

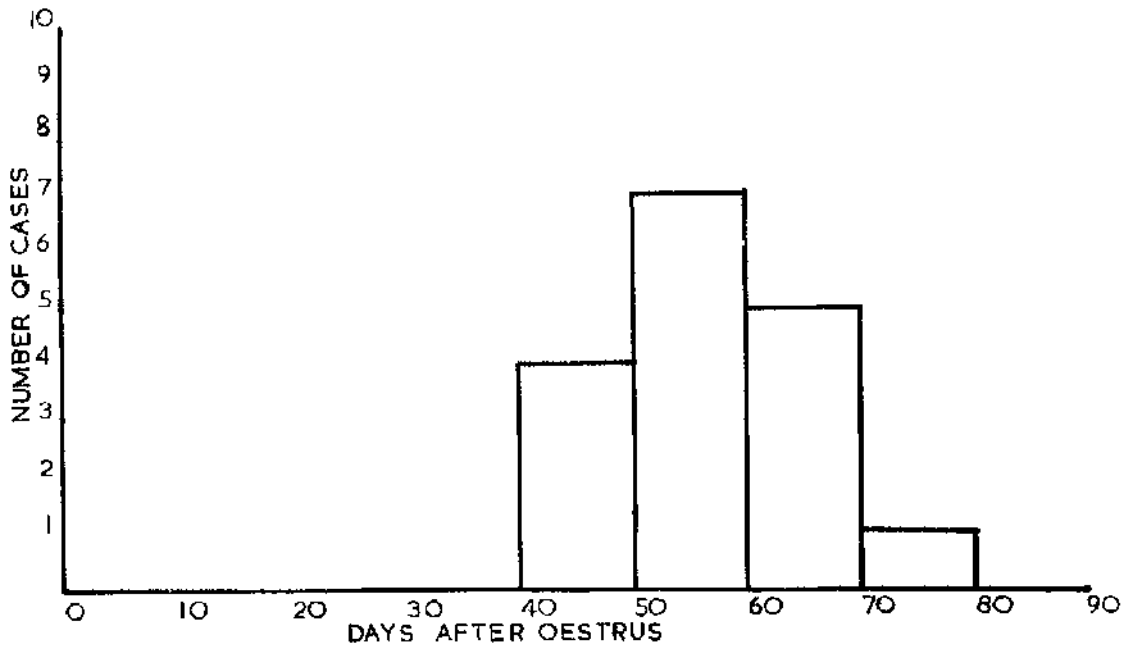
Thirteen bitches had never been pregnant. The other four had each borne a single litter between five and nine years prior to examination. It was ascertained that at least nine of the maiden bitches had never been mated. A history of phantom pregnancy was discovered in four animals. One bitch had exhibited signs of phantom pregnancy during each cycle for more than three years.

Oestral History.

The characteristic lesion of this group was found only in animals in the period 40-70 days after oestrus. The incidence of stage of cycle is shown in Fig. 16. Irregularities in the cycle were recorded in nine animals.

Fig.16

Incidence of stage of cycle in cases of cystic glandular hyperplasia associated with plasma cell infiltration.



Clinical Signs

The presenting sign was a glairy, mucoid, vulval discharge. Discharge had commonly been present for several weeks; in some cases, the owner was certain that the vaginal flow had not ceased after oestrus but had continued throughout metoestrus. In most cases, the discharge had been intermittent. Occasional bouts of anorexia and malaise had been noted. In two cases, the animal had vomited several times some two to three weeks before admission. The only evidence of abnormality detected on physical examination of these animals was discharge and oedema of the vulva. Abdominal distension was not observed in any animal.

Radiography

Ten cases were submitted to radiographic examination and in nine of these, the uterus was recognisably enlarged.

Haematology

Oxalated samples of blood were taken from 15 cases. There was no evidence of anaemia. Total leucocyte counts ranged from 12,000 to 15,000 per cu. mm. but differential counts were within normal limits.

Biochemistry

Plasma urea levels were estimated in 12 cases. Increased urea concentrations were detected only in cases with intercurrent renal disease.

Serum bilirubin levels were estimated in nine cases. No abnormality was observed.

Serum calcium, inorganic phosphate and protein levels and albumin/globulin ratios were normal in the five cases examined.

Vaginal Cytology.

Vaginal smears were obtained from 16 cases. Differential cell counts were compatible with the stage of cycle. In a few cases, there was an increase in the number of neutrophils.

Morbid Anatomy.

The uteri were generally enlarged but the diameter of the horns rarely exceeded two cm. The increase in diameter was referable in most cases to hypertrophy of the uterine walls. The uterine lumina contained a small amount of mucoid fluid which was usually clear but occasionally slightly brown or pink. In two cases, there was between 40 and 50 ccs. of this fluid. The cervical canal in every instance permitted passage of a probe of at least 0.3 cm. diameter and organic lesions of the cervix were not observed. The endometrium was a dull grey colour and the cysts were less obviously transparent than those in uncomplicated cystic glandular hyperplasia.

Histology.

The basic endometrial picture is that of cystic glandular hyperplasia with infiltration of the surrounding stroma by plasma cells (Fig. 17).

The superficial epithelium is of the florid, highly secretory type with a marked tendency to form pseudo-papillomatous proliferation. The epithelial proliferation appears as small fan-shaped tufts of cells or as broad sheets of up to ten cells thick extending into the uterine lumen. The nuclei of cells on the luminal edge of these sheets are hyperchromatic and pyknotic, and in some cells, the nucleus has disappeared leaving a completely vacuolated cell in the form of an intra-epithelial cyst. An occasional lymphocyte or neutrophil may be seen in the epithelium. Many of the superficial cysts are lined by a similar epithelium which gives a scalloped edge to their lumina. In such cysts, the cytoplasm often extrudes from the surface of the individual cells in the form of small papillae. The cysts of the basalis zone of the endometrium are generally smaller and of more uniform diameter. They are lined by a low columnar or cuboidal epithelium the cytoplasm of which rarely contains secretory vacuolation. All the cysts contain a variable amount of secretion which is commonly cell-free. The cellular infiltration of the endometrium is almost entirely composed of plasma cells with a few lymphocytes, and macrophages and an occasional mast cell (Fig. 18). Where the plasma cell infiltration is concentrated in the superficial area of the endometrium, the stroma of the basalis zone is oedematous and composed of fine collagen fibres with occasional scattered stromal and plasma cells. In many such cases, the closely packed cysts reduce the basalis stroma to a few fibres compressed between the walls of adjacent

cysts. In other cases, there is diffuse plasma cell infiltration of all layers of the endometrium. The basal endometrium is always highly vascular and moderately large vessels can be seen extending towards the uterine lumen. The superficial capillaries are dilated but there is no evidence of haemorrhage though a few haemosiderin-containing macrophages are scattered throughout the stroma. There is no necrosis or atrophy of any endometrial structure. In 14 cases, there is no fibroblast proliferation nor is there change in number, type or arrangement of collagen fibres. In two cases, there is a moderate increase in the number of fibroblasts in the tubular and crypt zones. The reticulin network of the crypt zone in these two specimens is less prominent and the collagen fibres are coarser and more numerous. In the final case, the most prominent feature is the distinct circular orientation of the collagen fibres of the superficial endometrium. The collagen fibres are thicker and more numerous than in the other cases. The reticulin network around the crypts and superficial cysts has been largely replaced by collagen but the basement membrane is still visible. The stroma of the basalis zone in this case is oedematous and shows no evidence of fibrosis.

The inner circular layer of the myometrium is hypertrophied and there is often some oedema of the stratum vasculare, the vessels of which are dilated. The perivascular connective tissue of the inner muscle layer contains a variable number of plasma cells forming cuffs round the vessels. This perivascular infiltration of the myometrium is commonest where the

plasma cell reaction in the endometrium is diffuse and intense.

In a few cases, perivascular infiltration by plasma cells is present in the stratum vasculare but it is not evident in the outer myometrium (Fig. 20). In three cases, adenomyosis is present in the stratum vasculare.

The cervix was obtained for histological examination in seven cases. In these, the cervical mucosa is moderately oedematous but cellular infiltration is slight. The most numerous cells are plasma cells but a few lymphocytes and neutrophils are evident in the surface epithelium and the immediately subjacent connective tissue. There is no evidence of fibrosis of the cervical mucosa or muscle layers.

GROUP III

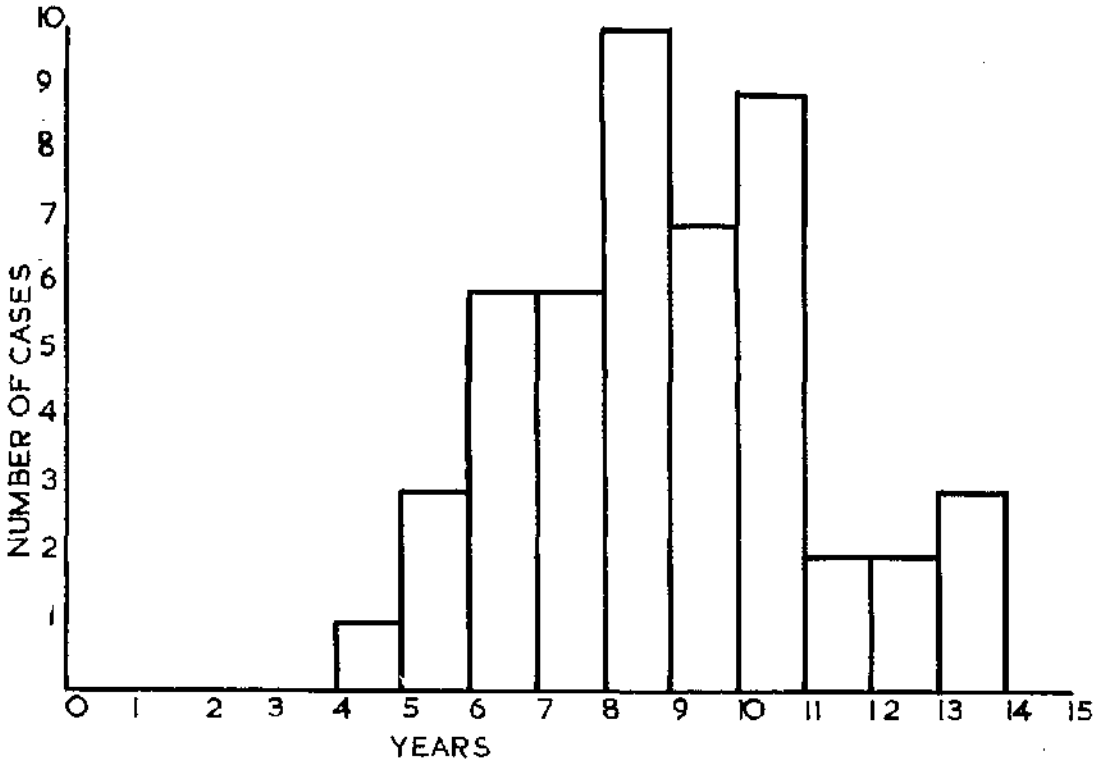
There are 49 cases in this section in which the characteristic lesion is an acute inflammatory reaction superimposed on a variable degree of cystic glandular hyperplasia of the endometrium.

Age Incidence

Full details of the age incidence of affected animals is shown in Fig. 21. The bitches were from four to 13 years old with a mean age of 8 ± 2.2 years. Only one case was under five years of age and the peak incidences were at eight years and at ten years.

Fig. 21.

Age incidence of cases of acute endometritis.



Breeding History

No record was available in three cases because of change of ownership. Thirty-eight were maiden bitches and, of the others, only three were multiparous. The interval between pregnancy and the onset of symptoms ranged from five to 11 years. It was ascertained that 21 cases had never been mated. Mating had occurred in three animals in recent years without conception. Five animals had a history of phantom

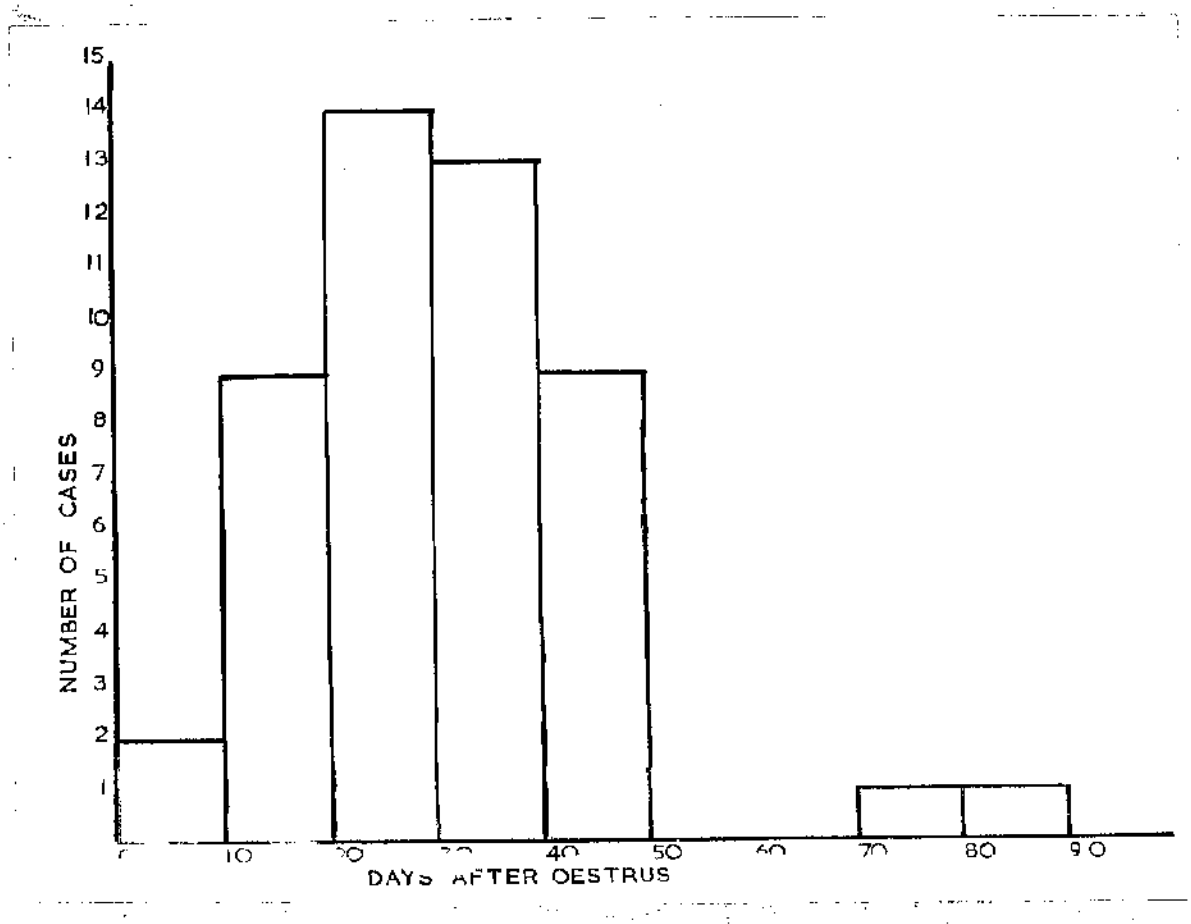
pregnancy.

Oestral History

The average interval between oestrus and examination was 30 ± 14 days and, though the range was five to 80 days, it is significant that 77% of the animals were presented for treatment in the first 40 days of metoestrus. The incidence of stage cycle is shown in Fig. 22. Fourteen animals had previously shown irregularities in the duration of oestrus or in the length of cycles. One 11 year old animal had shown regular normal oestrus periods once a year since puberty. There was a history of vulval discharge and sometimes periods of inappetance during previous cycles in seven cases.

Fig. 22.

Incidence of stage of cycle in cases of acute endometritis.



Clinical Signs

When presented, all the animals showed evidence of dullness and listlessness. It was commonly found that the duration of the present illness was from four to seven days. In six cases, the animal had become ill some three or four weeks previously, had improved and had relapsed just prior to admission. Vulval oedema was present in almost every case. A green, brown or bloodstained muco-purulent vulval discharge was evident in 38 animals. It was ascertained that discharge was intermittent in approximately one-third of the group. Some had discharged for several weeks and then ceased with subsequent increase in severity of illness. Others had discharged for only a few days before becoming clinically ill. A minority had never shown evidence of discharge, but several of these commenced to do so while awaiting surgical treatment. Increased thirst had been noted in 32 cases. Incontinence was frequently observed in association with polydipsia. Vomiting was observed in 21 animals at some time during the course of the illness. It was sometimes noted that vomition occurred only at the start of the illness when inappetance was first observed and did not occur again. Vomiting was most frequent and most severe in the presence of gross uterine distension. Some degree of abdominal distension was seen in 40 cases, usually accompanied by increased tension of the abdominal muscles except in completely prostrate animals (Fig. 23). Diarrhoea was not a common symptom and was observed in only four cases.

The body temperature showed considerable variation. In 38 cases the temperature was within normal limits, in eight it was from 103°F to 104.5°F and in the remaining three it was under 100°F. As a general rule, those cases which were discharging profusely had normal temperatures whilst those in which discharge was absent were either above or below normal.

The pulse rate was usually accelerated and the volume was good except in prostrate animals and in those with concurrent nephritis.

Haematology

No quantitative or qualitative abnormality was observed in the red cells. Total leucocyte counts ranged from 19,000 to 145,000 cells per cu. mm. Where vulval discharge was absent, the total white count was always over 40,000 but very high figures were also obtained where there was marked extension of the acute inflammatory process to the myometrium. The leucocytosis was always due to increase in the number of neutrophils, of which up to 35% were immature forms. The immature neutrophils were generally of the non-lobulated variety but in some cases myelocytes formed up to 2% of the total. In a number of cases, abnormal features were evident in the morphology of the neutrophils, particularly, in the non-lobulated forms. The most common change was increased cytoplasmic basophilia with a tendency to form irregular, more deeply staining granules. The cytoplasm of some cells was irregularly streaked with

deeper blue end in others, vacuoles were evident. In supravitally stained smears, many of the non-lobulated neutrophils contained an abnormally low complement of granules. Cellular fragility appeared to be increased and there were large numbers of "smudge" cells in many blood films.

Erythrocyte counts and haemoglobin concentrations were within normal limits in all the cases examined.

The erythrocyte sedimentation rate was constantly accelerated to between ten and 55 mm. per hour and was used as an aid to prognosis. Rates of more than 30 mm. per hour were associated with high surgical mortality or with slow post-operative recovery.

Table 5

Details of haematological findings in five cases of acute endometritis

Details of haematological findings in five cases of acute endometritis

	Case 59	Case 71	Case 63	Case 89	Case 99
Erythrocytes	6.95×10^6	8.30×10^6	7.05×10^6	7.90×10^6	8.55×10^6
Haemoglobin	12.4	15.2	14.0	14.9	15.5
Total leucocytes	125,200	23,600	37,000	63,050	29,350
lobulated Neutrophils	56%	71.5%	65.5%	72%	73%
Non-lobulated Neutrophils	33%	5%	13%	19%	4%
Myelocytes	1%	0	0.5%	1%	1%
Eosinophils	1%	1.5%	2%	0.5%	2%
Lymphocytes	4.5%	13.5%	16%	4.5%	16.5%
Monocytes	4.5%	3.5%	3%	3%	3.5%

Biochemistry

Plasma urea levels were determined in 38 cases and were used as an aid to prognosis. The average level was 37 ± 9 mg. per 100 ml. with a range of 21 to 119 mg. It was found that animals with high levels were poor operative risks. Of ten animals with urea levels over 55 mg. per 100 ml. four died during ovaro-hysterectomy or in the immediate post-operative period. In the other six with high urea concentrations, convalescence was prolonged. Agglutination tests for Leptospira canicola were not performed as a routine so it was impossible to determine whether uraemia was due to genital disease or to concurrent nephritis. It is significant that all four animals which died showed renal lesions typical of L. canicola infection.

Serum bilirubin levels were estimated in ten cases. The levels were within normal limits in eight cases and slightly increased in two. Full details are given in Table 6.

Serum calcium and inorganic phosphate concentrations were determined in ten cases. As shown in Table 6, the levels were within normal limits.

Serum protein estimation was carried out in ten cases. In every case, there was an increase in the serum protein level which ranged from 7.1 to 10.2 gm. per 100 ml. The albumen/globulin ratios were reduced in all cases indicating an increase in the globulin fractions.

Table 6

Details of biochemical findings in ten cases of acute endometritis

Case	Urea (mg/100 ml)	Bilirubin (mg/100 ml)	Calcium (mg/100 ml)	Inorganic Phosphate (mg/100 ml)	Total Protein (g/100 ml)	A/G Ratio
S 59	119	0.7	9.1	3.3	7.1	0.65
S 63	31	0.1	10.0	3.7	7.8	0.35
S 71	74	0	9.9	3.4	8.9	0.3
S 72	37	0.1	10.5	4.9	7.9	0.5
S 77	25	0.2	10.1	5.2	8.6	0.4
S 79	62	0.9	10.6	4.7	10.2	0.3
S 83	37	0.1	10.4	4.9	9.7	0.35
S 86	41	0.3	11.1	5.1	8.9	0.4
S 91	51	0.1	9.2	3.8	10.0	0.35
S 94	29	0.2	9.9	4.1	9.2	0.3

Radiography

Diagnosis was generally confirmed without difficulty on radiography without the necessity of resorting to pneumoperitoneum or any other device to obtain contrast. Diagnosis on a purely radiographic basis was

difficult in those cases in which the uterus formed a series of ampullae. In these, the resemblance to the normal uterus in the latter half of pregnancy was striking.

Vaginal Cytology

Vaginal smears were obtained from 43 cases and the average differential cell counts are given in Table 7. Some difficulty was experienced in cleansing the vagina of fluid discharged from the uterus. The differential cell counts cannot be considered to give a true reflection of the vaginal cytology because of the unavoidable contamination by uterine fluid. Differential cell counts were similar in open and in closed cases though the former inevitably contained more neutrophils. Erythrocytes were present in many smears and were presumed to be of uterine origin.

Table 7

Vaginal Cytology in Acute Endometritis

Cell Type	5-20 days post-oestrus	20-40 days post-oestrus	Over 40 days post-oestrus
Confined Epithelial	23%	0%	0%
Eosinophil Epithelial	49%	39%	42%
Small Basophil	28%	43%	49%
Large Basophil	0	18%	9%
Erythrocytes	Variable	Variable	Variable
Neutrophils	++++	++++	++++

Morbid Anatomy

The size of the uterus varied inversely with the degree of patency of the cervix. The cervical canal was completely closed in eight specimens and in a number of others, the os uteri was narrow, allowing discharge of only a fraction of the fluid. The uterine horns were generally more than 2 cm. in diameter. Uteri weighing up to four kilogrammes and with horns measuring up to 50 cm. in length and up to seven or eight cm. in diameter were not uncommon in bitches weighing between 15 and 20 kgs. (Fig. 24). Where the horns were grossly enlarged, they were forced into coils by the restraining action of the broad ligament. The horns might be of uniform diameter throughout their length but more often they exhibited a number of annular constrictions producing a series of irregular ampullae. The constrictions were usually slight but in some cases, they were so marked that a series of non-communicating ampullae were found. The corpus uteri rarely showed the same degree of distension as the uterine horns and, in some, it appeared of little more than normal diameter. The cervix was never enlarged except in cases where it was relaxed. In three specimens, distension was confined to one horn. The contralateral horn was hypertrophied or of normal proportions. In most of the uteri examined, the walls were considerably increased in thickness. In grossly distended specimens, decrease in thickness of the wall was evident. Most of the increase in thickness appeared to be due to endometrial rather than myometrial hypertrophy

though the latter was obvious in some freely discharging cases. It was observed that the uterine wall was frequently thicker at the points of constriction and small abscesses were not uncommonly present at these points.

The volume of fluid in the uteri ranged from a few ml. up to 2.5 litres, depending on the adequacy of cervical drainage. The fluid was yellow or green in some cases but more frequently it was tinted brown or red by extravasated blood. The viscosity of the fluid varied considerably; it was usually lowest when haemorrhage was present and highest where the fluid was of a yellow-green colour. Massive intra-uterine haemorrhage was not observed in any of the specimens examined.

The endometrium had a roughened appearance with mingling of pus-filled cysts, focal haemorrhages and areas of ulceration. Thick muco-pus was often adherent to areas of ulceration. Ulceration was prominent in grossly distended uteri. In tightly constricted segments and in the unaffected horns of unilateral cases, the endometrial surface sometimes had the appearance of uncomplicated cystic hyperplasia. In no case was there evidence of organic lesions in the cervix.

Localised areas of peritonitis were noted in eight uteri and in one case, an acute peritonitis had followed rupture of the uterine wall.

Bacteriological Examination

The uterine fluid from every case was submitted to cultural examination. Only five cases failed to yield bacterial growth. B. coli

was the commonest organism isolated, occurring in 40 cases. Six cases yielded a coagulase-positive staphylococcus and five contained a B-haemolytic streptococcus. It is significant that bacteria were isolated from six specimens in which the cervix was completely closed and from two of three unilateral cases. No organisms were isolated from the unaffected horns of unilateral cases.

Histology

The characteristic feature of this group is the presence of an acute inflammatory reaction in an endometrium which shows a variety of stromal and glandular changes. In most specimens, the cystic glandular hyperplasia is divided into two morphologically distinct layers, superficial and basal (Fig. 25). The superficial cysts are very irregular in outline and are lined by an epithelium which, in general, is similar to that of the crypts and endometrial surface. The epithelial cells are tall and of a secretory type. They may be in a single layer but, often, the arrangement is more complex. Pseudo-stratification may be focal, producing numerous small epithelial papillae or it may be diffuse (Fig. 26). Sometimes, the epithelium forms syncytium-like sheets in which cell boundaries are indistinct and vacuolation of cells is prominent. Degenerative changes in the epithelium are seen where proliferation has been most active. The cells lose their staining affinity and the nuclei are hyperchromatic and pyknotic. The basal cysts are always more

regular in outline. They are lined by a simple cuboidal epithelium in most instances though occasional foci of cysts lined by tall, secretory cells are seen. The lumina are usually filled with secretion.

In mild cases, cellular infiltration is confined to the superficial half of the endometrium. Numerous neutrophils are seen in the stroma beneath the superficial epithelium and around the crypts and adjacent cysts. Infiltration of the epithelium is particularly prominent in areas of stratification and the uterine lumen contains large numbers of neutrophils mixed in the secretion. Inflammatory exudation into the crypts and cysts is irregular and whilst some contain only mucin others are filled with neutrophils and cell debris. Necrosis of some of the small endometrial polypi is seen at this stage. All the superficial capillaries are widely dilated and some degree of stromal oedema is present (Fig. 27). The basal half of the endometrium shows no evidence of neutrophil infiltration. The stroma is very oedematous and congestion of the basal vessels is evident.

In more severely affected cases, the neutrophil infiltration extends into the basal half of the endometrium. Many cysts become completely filled with inflammatory exudate and degenerative changes are visible in the lining epithelium (Fig. 28). Haemorrhage into individual cysts and into the basal stroma is seen in some cases. Abscesses are frequently formed around cysts in which the lining epithelium has undergone necrosis. These basal abscesses may extend to occupy the

entire width of the endometrium and may ulcerate into the uterine lumen. When these abscesses rupture, they appear to become confluent with the uterine exudate (Fig. 29).

Ulceration of superficial epithelium is not always associated with rupture of basal abscesses and typical acute erosions may be seen with necrosis of subjacent vessels and haemorrhage into the uterine lumen. In many grossly distended uteri, ulceration is widespread and complete sloughing of the entire endometrium may be seen in parts. Ulceration may also be seen in association with thrombosis of some of the basal arteries. In the base of small isolated ulcers, there is sometimes evidence of early granulation tissue formation.

Plasma cells are present in every specimen examined in this group. Their number and distribution shows no relation to the neutrophil infiltration but appears to be similar to the lesions described in Group II. In a few cases neutrophils are present, only in the epithelium and in the uterine and cyst lumina whilst the stromal infiltration is entirely composed of plasma cells. In many cases, eosinophils are present but never in large numbers.

Evidence of fibrosis is generally absent but it is present in five cases, three of which are known to have had previous attacks of post-ocstral endometritis. In all five cases, it appears as a broad, circular band of connective tissue. In two specimens, the fibrous tissue which contains only a few glandular lumina lies between the

superficial and basal layers of cysts. The superficial zone is diffusely infiltrated by neutrophils but the area beneath the fibrous tissue shows no inflammatory reaction (Fig. 30). In the other cases, there is only a narrow lamina propria of relatively fine connective tissue separating the zone of fibrosis from the superficial epithelium which is composed of low cuboidal cells. There is intense neutrophil infiltration of the lamina propria and the subjacent fibrous tissue with ulceration at many points. The basal zone shows cystic glandular hyperplasia, hyperaemia and oedema.

In 50% of the uteri examined, there is extension of the acute inflammatory process to the myometrium. Where ulceration is severe, there is necrosis of muscle fibres and diffuse neutrophil infiltration of the myometrium in the base of the ulcer (Fig. 31). These ulcers rarely extend far into the inner, circular layer of the myometrium; in only one case has complete perforation occurred. The inner muscle coat is frequently involved in abscesses arising in the basal zone of the endometrium. The commonest type of myometrial involvement is extension of the inflammatory reaction along the perivascular connective tissue. The vessels penetrating the inner myometrium are commonly surrounded by cuffs of neutrophils and plasma cells. The stratum vasculare is always oedematous and the vessels in it are dilated and show increased tortuosity as in oestrus. The lymphatic vessels are distended and contain large numbers of polymorphonuclear leucocytes. Abscesses of the stratum

vasculare are not uncommon and infiltration of the walls of neighbouring vessels is sometimes seen. Thrombosis of these vessels is present in one instance. It is of interest that one specimen shows typical lesions of polyarteritis nodosa in the arteries of the uterus, ovaries and broad ligaments in association with a mild acute endometritis. Muscle biopsies (taken at a later date) reveal that the condition was not generalised. In some specimens, there is an increase in the amount of connective tissue in the stratum vasculare and in the outer longitudinal layer of the myometrium. The outer myometrium is hypertrophied in most cases. Where uterine distension is gross, degenerative changes in the muscle fibres are evident. There is atrophy of muscle fibres with loss of staining affinity.

The lamina propria of the cervix is usually oedematous and contains a mild cellular infiltrate composed largely of plasma cells with a few neutrophils and lymphocytes. In a few cases, an acute cervicitis is present. Evidence of fibrosis cannot be found in any specimen.

GROUP IV

This is the smallest group in the series and is composed of 11 cases in which the characteristic lesion is a chronic endometritis.

Age Incidence

The animals in this group were significantly older than those in

the other groups in the series. They ranged from 9-15 years of age with a mean of 11.8 ± 1.7 years. The peak incidence occurred at 11 years and at 12 years. The age incidence of affected animals in the series is shown in Fig. 32. Since completion of the 100 cases in the survey, a case of chronic endometritis has been found in an animal of six years old.

Breeding History

Definite information was obtained in nine cases. Of these, seven were maiden bitches. The other two had both whelped twice between six and nine years prior to examination. It was believed that seven bitches had never been mated. One animal had been mated at each of the two preceding oestrus periods but had failed to conceive. Phantom pregnancy had been observed in two cases.

Oestral History

These bitches were admitted to the hospital between 55 and 85 days after oestrus with a peak incidence between the 60th and 70th days. Nine cases had a history of irregular oestrus cycles and of previous bouts of metoestral genital disease.

Course and Symptoms

As stated in the previous paragraph, nine of the bitches in this group had a history of previous post-oestral genital disease. The

owners' recollections were essentially vague but all indicated a close similarity between the present illness and previous attacks. Most of the owners had noted vulval discharge during metoestrus periods and some had come to accept this as a normal event. Four cases had received medicinal treatment of some type for previous attacks of endometritis. It was not possible to discover the exact nature of this treatment.

The duration of the present illness varied considerably but it could generally be traced back to the last oestrus period. Vulval discharge had commenced shortly after regression of oestrus and had been continuous or intermittent since that time. Some had shown several periods of a few days illness followed by profuse discharge during metoestrus. Others had discharged until 5-10 days before examination and had then become seriously ill.

Fig. 32.

Age incidence of cases of chronic endometritis.

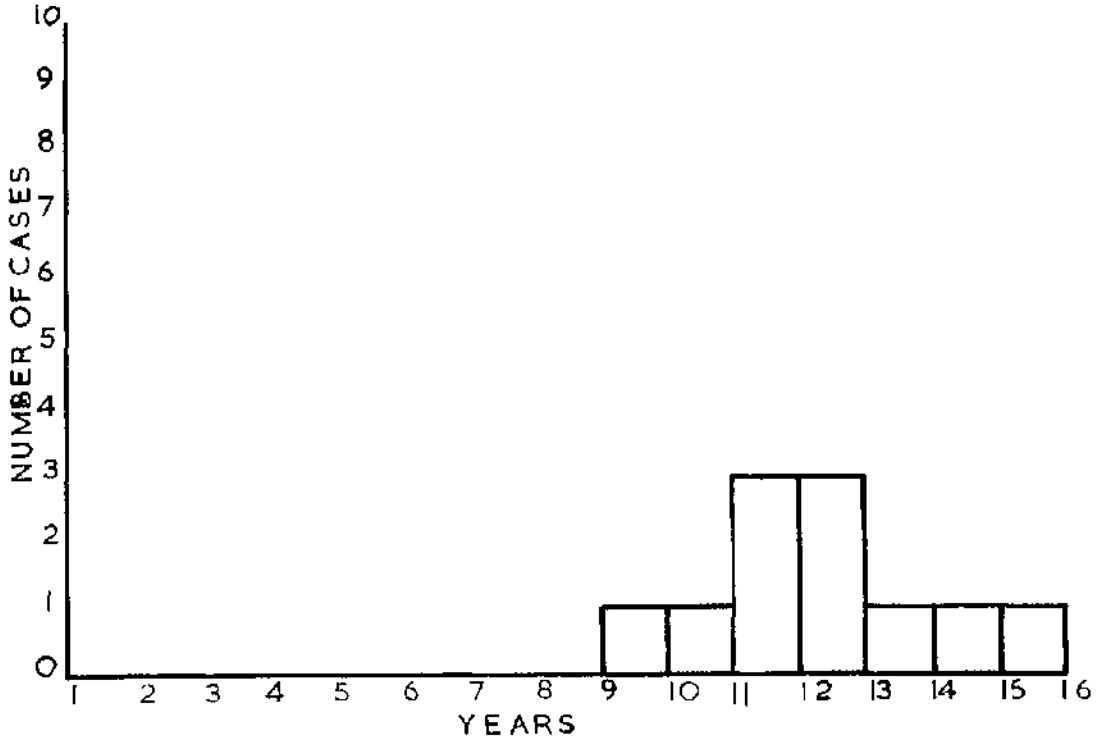
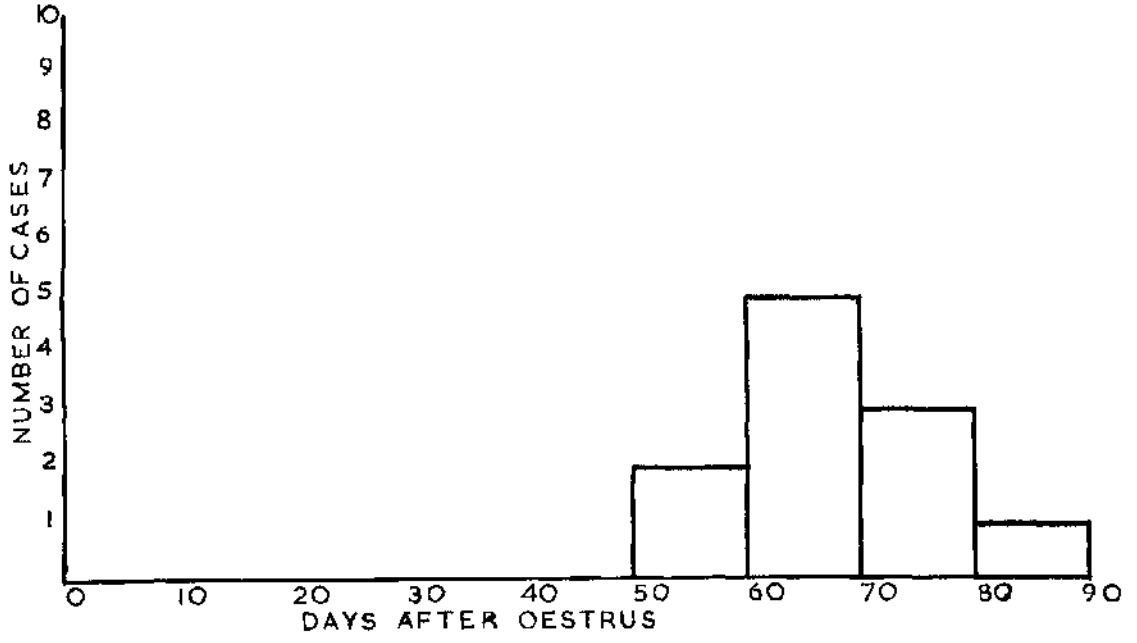


Fig. 33.

Incidence of stage of cycle in cases of chronic endometritis.



On physical examination, these cases were divided into two groups depending on the presence or absence of vulval discharge. In four bitches, there was little systemic upset and the only detectable abnormalities were vulval oedema and discharge. The other seven cases were seriously ill and four of them were in a state of collapse. There was considerable abdominal distension and vulval oedema but discharge was absent. They exhibited increased thirst and vomited frequently. Body temperatures in these seriously ill animals was generally above normal limits except in prostrated animals where subnormal temperatures were

noted. The pulse rate was accelerated and of poor volume. In both types, skin changes were common. The skin was dry and inelastic with areas of alopecia on the flanks, over the abdomen and on the perineal region. In hairless areas, the skin was roughened and appeared similar to that seen in low-grade chronic eczemas.

Radiography

The diagnosis of pyometra in cases with cervical occlusion was readily confirmed by radiography but in chronic discharging cases it was necessary to induce artificial pneumo-peritoneum to obtain contrast.

Haematology

No evidence of anaemia was found in either type. In the clinically mild cases, total leucocyte counts ranged from 16,000 to 21,000 per cu. mm. and in the severe cases, from 31,000 to 68,000 per cu. mm. The leucocytosis was largely due to an increase in neutrophils but in some cases, bizarre mononuclears accounted for up to 7% of the total white count. These cells were approximately 20 microns in diameter and had irregularly stellate nuclei. The nucleus which possessed up to ten lobes contained relatively little chromatin and had the loose structural appearance of a monocyte nucleus. The cytoplasm was a dull blue with occasional vacuoles in it. No cytoplasmic granules were observed in Leishman or supravitally stained smears. Such cells have been observed in other chronic conditions in dogs admitted to the hospital.

The erythrocyte sedimentation rate varied from 8 to 15 mm. per hour in mild cases and from 25 to 40 mm. per hour in severe cases.

Biochemistry

Plasma Urea Concentration. Urea levels, estimated in nine cases, ranged from 29 to 47 mg. per 100 ml. of blood. Levels were highest in the severely ill animals but since agglutination tests for L. canicola were not done it is impossible to say whether uraemia was associated with chronic endometritis or with a concomitant nephritis.

Serum Bilirubin levels were determined in six cases and were within normal limits in five cases. In the sixth case, the bilirubin level was 2 mg. per 100 ml.

Serum Protein estimations were performed in six cases. The levels ranged from 7.2 to 9.4 g. per 100 ml. There was an increase in the globulin fractions with a fall in the albumen/globulin ratios. Details are given in Table 8.

Serum Calcium and Inorganic Phosphate levels were within normal limits in the six cases in which estimations were carried out.

Table 8

Details of Biochemical findings in six cases of chronic endometritis

Case	Urea (mg/100 ml)	Bilirubin (mg/100 ml)	Calcium (mg/100 ml)	Inorganic Phosphate (mg/100 ml)	Total Protein (g/100 ml)	A/G Ratio
S 64	29	0.1	8.6	3.4	9.4	0.35
S 69	35	0.2	9.1	3.9	8.6	0.4
S 84	41	0.3	8.5	4.6	7.4	0.6
S 87	34	2.0	10.3	5.2	7.2	0.55
S 92	46	0.3	10.0	4.7	8.1	0.4
S 95	39	0.4	9.7	4.3	9.2	0.4

Morbid Anatomy

The uteri were divided into two pathological sub-groups corresponding to the clinical subdivision.

In the seriously ill animals, the cervix was tightly constricted so that the os was completely occluded. There was no obvious enlargement of the cervix. The uterine horns were grossly distended and the walls were of paper thinness so that it was impossible to differentiate endometrium and myometrium (Fig. 34). The uteri contained up to

2.5 litres of thin, watery brownish fluid. Highly viscid muco-pus was not observed in this form of the cystic hyperplasia-pyometra complex. The endometrial surface was greyish with occasional flecks of haemorrhage and had a finely granular appearance. At intervals, low irregular ridges were apparent on the endometrium but no cysts or polypi were observed. Localised plastic peritonitis was present in two cases but generalised peritonitis was not observed at ovaro-hysterectomy.

In the mild cases, the cervix was relaxed and the canal was open. The uterine horns though often elongated, were never more than 3 cm. in diameter. In two cases, the uterine walls were of little more than normal thickness but in the other two, they were grossly hypertrophied. In the latter, increase in thickness appeared to be of myometrial rather than of endometrial origin. The uterine lumen was small and rarely contained more than a few millilitres of viscid, yellow-green muco-pus. The endometrial surface was almost normal in appearance though occasional polypi were present. There was no evidence of endometrial ulceration or haemorrhage in these specimens.

Bacteriological Examination

Samples of uterine fluid from all cases were submitted to cultural examination. In two cases in which the cervical canal was occluded, no organisms were found. B. coli was obtained from eight uteri, Staph.

aureus from two and a B-haemolytic streptococcus from two.

Histology

Though the microscopic picture is one of chronic endometritis in both sub-groups, there are differences in the histopathological processes which are as distinct as the clinical and morbid anatomical features.

In the distended uteri, only a thin lamina propria separates the superficial epithelium of the endometrium from the inner layer of the myometrium. This lamina propria is slightly thicker in sections taken from the corpus uteri and from the endometrial ridges mentioned above. In four specimens, the superficial epithelium is a low cuboidal or pavement type which is highest in the corpus uteri where distension is least. The cytoplasm of these cells is pale eosinophilic and homogeneous with no evidence of secretory vacuolation. The nuclei are small and hyperchromatic (Fig. 35). In one of these cases, sections taken from the corpus uteri and the upper ends of the horns show occasional areas of stratification suggestive of squamoid change. In the other three cases, the superficial epithelium is of a stratified squamous type (Fig. 36). This stratified epithelium varies from five to 12 layers thick. The basal cells are elongated and at right angles to the surface and have well defined cell borders. There is commonly a thin stratum granulosum but the most of the epithelium is of a

cornified type which still possess flattened deeply staining nuclei. There is a striking similarity between this epithelium and the normal vaginal epithelium in early metoestrus. Crypts are relatively scanty except in the corpus uteri and in endometrial ridges and are always lined by an epithelium similar to that of the surface. Glandular elements are present in moderate numbers at the endometrial thickenings and in the corpus uteri; elsewhere there are only isolated lumina. Occasional glands extend into the inner layer of the myometrium. The epithelium lining these glands is often of a tall secretory type but in two cases, squamoid metaplasia has extended to the glands. In one of the latter cases, stratified squamous epithelium is also present in an adenomyotic focus in the stratum vasculare (Fig. 37). The thin lamina propria is composed of closely packed coarse collagen fibres arranged in a circular manner parallel to the surface. The reticulin network of the endometrium has been replaced by collagen fibres except where it forms a basement membrane for the superficial and glandular epithelium. There is diffuse lymphocyte and plasma cell infiltration of the stroma in all but one case in which the lamina propria is almost cell-free. There are occasional small ulcers and neutrophils are present in relation to these. The bases of these ulcers contain highly vascular granulation tissue but in general, hyperaemia of the endometrium is not prominent. In one case, considerable numbers of neutrophils have infiltrated the stratified squamous epithelium.

Both the inner and outer layers of the myometrium are atrophic. The muscle fibres are thin and have lost their staining affinity. The nuclei are slender, elongated and hyperchromatic. In most specimens there is diffuse plasma cell and lymphocyte infiltration of the myometrium. The stratum vasculare is compressed between the two layers of muscle and is only identifiable as a number of isolated vessels. These vessels are small and have thin walls.

The cervical epithelium is normal in structure. The lamina propria is thickened and contains a small number of plasma cells and lymphocytes. The collagen fibres are coarse but there is no marked increase in number. They are arranged in a distinctly circular pattern. The muscularis of the cervix is hypertrophied but there is no evidence of fibrosis.

In the mild cases, where the cervical canal was patent, the endometrium is atrophic. The superficial epithelium is composed of low cuboidal cells or of the fatty, degenerate type normally seen in late metoestrus. Crypts are few and lined by epithelium similar to that of the surface. The endometrium is narrow and rarely more than two glandular lumina in width. The glands are commonly small and atrophic with a low cuboidal epithelial lining. Occasional polypoid cysts are present but most of the small superficial papillomata do not contain glandular elements (Fig. 38). There is a mild diffuse infiltration of the endometrium by plasma cells and lymphocytes. Some increase in

number of collagen fibres is evident, particularly round the glands and in the superficial endometrium. The fibres are coarse and have replaced the sub-epithelial reticulin net-work leaving only a thin basement membrane.

In the myometrium, there is an increase in the amount of connective tissue separating the muscle bundles. This connective tissue is less cellular and is composed of coarse collagen fibres. This change is particularly prominent in the outer layer of muscle which is usually considerably thinner than the inner layer. In those cases where myometrial thickening was distinct at biopsy, the muscle fibres are hypertrophied. In the others, there is atrophy of the muscle fibres and increased fibrosis. The walls of the vessels of the stratum vasculare are thickened and the lumina propria relatively small. Perivascular cuffing by lymphocytes and plasma cells is marked in the vessels traversing the annular myometrium.

The cervical mucosa is infiltrated by lymphocytes and exhibits connective tissue changes similar to those in the endometrium.

The Histochemical Examination of Endometrial Mucin in the Cystic Hyperplasia-Pyometra Complex.

The staining reactions of the endometrial mucin in the four groups is shown in Table 9. Comparison with Table 1 shows no detectable difference in composition of the mucin in the normal and in the diseased uterus. In the normal animal, mucin is present in the form of intra-cytoplasmic droplets only during oestrus and the first fortnight of metoestrus. In many cases of the cystic hyperplasia-pyometra complex, intra-cytoplasmic mucin is present in both the superficial and glandular epithelium as late as 50 days after oestrus. This phenomenon is particularly common in those cases of acute endometritis occurring after the 30th day of metoestrus. Intra-cytoplasmic mucin is constantly found in cases in which the cervix is closed. Where the cervix is open in Groups II and III, intra-cytoplasmic mucin is less abundant but the amount in the uterine and glandular lumina is suggestive of excessive secretion (Fig. 39). In Group IV, mucin is present only in the lumina of the few remaining glands and its staining intensity is less marked, probably because of dilution with inflammatory exudate. Sudanophilic droplets are present in the superficial epithelium in some of these chronic cases but this is a normal feature of late metoestrus. No evidence of mucin was found in the inflammatory exudate in the uterine lumen in these cases where squamoid metaplasia had

occurred in the lining epithelium.

Table 9

Histochemistry of Endometrial Mucin in the Cystic Hyperplasia-Pyometra Complex

Staining Reaction	Group I	Group II	Group III	Group IV
P.A.S.	+	+	+	+
Diastase + P.A.S.	+	+	+	+
Toluidine Blue	Metachromasia	Metachromasia	Metachromasia	Metachromasia
Methylene Blue Extinction	pH 2	pH 2	pH 2	pH 2
Southgates Mucicarmine	+	+	+	+
Alcian Blue	+	+	+	+
picro-Mallory	pale blue	pale blue	pale blue	pale blue
Sudan Black B	-	-	-	-

The Ovary in the Cystic Hyperplasia-Pyometra Complex.

No group specific ovarian changes were observed and a composite description is given.

Morbid Anatomy

Eighty-eight cases were in metoestrus and of these, 80 contained recognisable corpora lutea in at least one ovary. There was no evidence of increase in the total number of corpora lutea in the ovaries. The mean total for 88 cases was 7.6 and for the same number of normal animals it was 7.9. In many cases in the latter half of metoestrus, the corpora lutea appeared larger than those in the normal animal at a comparable stage in the cycle. In many cases, corpora lutea were suspended from the ovaries by slender pedicles. In seven cases, both ovaries were transformed into multiloculated cystic masses of up to 7 cm. long (Fig. 40). The cysts were thin-walled and contained a clear, watery fluid. Corpora lutea were not identified in these ovaries. In two cases, multicystic change was observed in only one ovary, the other contained corpora lutea. Single, thin-walled cysts of 1-3 cm. diameter were observed in a further eight specimens. In three cases, one ovary contained a solid, lobulated mass of 2-3 cm. diameter. These tumour masses were homogeneous, white and cellular in appearance. Corpora lutea were present in the contralateral ovary

in two cases and in the third, the ovary was small and smooth.

In those cases of uncomplicated cystic hyperplasia which were not in metoestrus, the ovaries were generally smooth and inactive. In two cases, both ovaries had undergone multicystic change. In one case, one ovary contained a tumour-like mass of 3 cm. diameter and the other ovary contained two single polypoid cysts of 2 cm. diameter.

Histology

Active or regressing corpora lutea are present in one or both ovaries in 96 cases. Personal observations show that in the normal animal the granulosa lutein cells reach their greatest size at about 10 days after ovulation and begin to undergo retrogressive changes after the 30th day.

In cases of the cystic hyperplasia-pyometra complex the corpora lutea appear consistent with the estimated stage of cycle up to about four weeks after oestrus. In clinical cases arising later in met-oestrus, it is common to find corpora lutea which have not undergone the expected degree of regression. There is only moderate connective tissue infiltration and the structure is highly vascular. Though some of the lutein cells are showing retrogressive changes, the majority appear of maximum size even in cases 50 days post-oestrus. These corpora lutea resemble those of pregnancy more closely than those of normal metoestrus in that the cytoplasmic lipids are still in discrete

droplets and have not become confluent. This apparent persistence of luteal function is particularly prominent in cases in Group III and in several of Group IV. There appears to be a correlation between the degree of persistence of the corpora lutea, the complexity of arrangement and cytoplasmic vacuolation of the endometrial epithelium and the degree of occlusion of the cervix. In Groups I and II, the corpora lutea are morphologically consistent with the stage of cycle.

The cysts which are present in 19 cases, are thin-walled structures lined by one or several layers of epithelium surrounded by remnants of the theca interna or by compressed ovarian stroma. In some single cysts, it is possible to recognise a lining of several layers of granulosa cells but, in most cases, there is only a single layer of low columnar or cuboidal cells. In the larger single cysts and in the polycystic variety, the lining epithelium is flattened and nuclei are dense and hyperchromatic or the epithelium may have disappeared and the cysts are lined by fibrous connective tissue. In one large solitary cyst, the lining is composed of several layers of granulosa cells for about three-quarters of the circumference and the remainder is lined by granulosa lutein cells which appear to be arising in the cumulus. Even in some ovaries of the polycystic type, in which only small islets and trabeculae of ovarian tissue remain, all stages of follicle growth and regression can be seen. In three polycystic ovaries no follicular or luteal structure can be found.

One unilateral polycystic mass proves to be a pseudomucinous cystadenoma lined by characteristic tall clear cells with dark-staining basal nuclei. The contralateral ovary contains morphologically functional corpora lutea.

Granulosa cell tumours are present in eight cases, though only four were considered abnormal at post mortem examination. In one case, the tumour is bilateral in site. The microscopic pattern is extremely variable even in any one tumour, though, in general, the individual cells closely resemble granulosa cells. In the majority, the micro-folliculoid pattern predominates with some areas of diffuse, solid growth. Call-Exner rosettes are more frequently observed in the microfolliculoid areas. In some parts, connective tissue ingrowth has produced a cylindromatous arrangement. In one case, more than one half of the tumour has undergone luteinisation whilst the remainder is of the microfolliculoid type.

In the normal canine ovary, the rete ovarii consists of a small group of anastomosing tubules situated in the hilar area and the adjacent part of the medulla. They are lined by a cuboidal or low columnar epithelium. The cytoplasm is palely eosinophilic and the dark, compact nuclei are situated in the basal half of the cell. In 26 cases, in the series, there is evidence of hyperplasia of these rete tubules to form a closely packed mass in the hilum. The epithelium is taller and pseudostratification is often seen. The change

is usually bilateral. This rete proliferation is present in less than 10% of normal ovaries examined.

Inflammatory changes in the ovaries are absent except in one case of polyarteritis nodosa. The utero-ovarian vessels in the hilar area are usually dilated and congested.

Discussion and Conclusions

This survey of the cystic hyperplasia-pyometra complex was undertaken to correlate the clinical, haematological, biochemical and pathological findings in the hope of shedding some light on the aetiology of the condition.

In the present series, the mean age of 100 cases is 8.2 ± 2.4 years. Though the range is from three to 15 years, only 4 animals are under five years of age. Since the life span of the dog is of the order of 10 years, it is obvious that the cystic hyperplasia-pyometra complex is a disease of later life. It is not directly comparable to the various post-menopausal diseases of women because the normal healthy bitch is able to breed throughout its adult life. This distribution of age incidence of the condition agrees with the observations of Benesch and Fommer (1930) who noted that the majority of their cases were between seven and 10 years old. Hesselbarth (1937) recorded a similar increased incidence of the disease between seven and nine years of age. Recently, Kummermann-Luscher (1952) has reported the occurrence of a pyometra in

animals of one year old. An accumulation of pus with serious illness has been observed in two one year old bitches admitted to this hospital. In one animal, the retention of pus was due to a congenital malformation of the corpus uteri and cervix and, in the other, to a large, oedematous fibroma of the ectocervix. In both cases, histological examination of the endometrium revealed an acute inflammatory reaction in an otherwise normal endometrium. It is possible that the cases reported by Kammermann-Luscher (1952) are of this type but lack of pathological evidence of uterine and cervical changes precludes any comparison with the condition discussed in the present work.

There is no correlation between the age of the affected animal and the degree of illness exhibited, though advanced age was commonly associated with high operative mortality or prolonged convalescence. The first three groups show no significant difference in age but the fourth group is considerably older. This is in keeping with the history of these chronic cases since all had shown previous attacks of endometritis.

It is significant that 76% of the animals examined had never whelped and, therefore, presumably had never been pregnant. From this, it may be inferred that absence of pregnancy is probably one of the underlying factors in the development of the disease. This conclusion is supported by the observations made in those cases which had whelped at some time prior to developing the disease. The shortest interval

between parturition and diagnosis of the condition was three years where only one litter had been borne. In multiparous bitches, the shortest interval increased to four and a half years and in most, it was more than six years. In early reports of endometritis in the bitch (Banham and Hobday, 1905, Bouchet, 1906, Charmoy, 1913), the high incidence of genital disease in maiden bitches aroused considerable surprise. Since no histopathological examinations were made, these workers failed to differentiate between puerperal metritis and the condition associated with cystic hyperplasia of the endometrium. Charmoy (1913) suggested that endometritis in the maiden bitch was caused by venereal infection and early death of the embryos. Of the 76 maiden bitches in the present study, 46 had never been mated and it is very unlikely that 25 of the remainder had been mated though the possibility could not be excluded with certainty. It can, therefore, be assumed that venereal infection is not an important factor in the aetiology of the cystic hyperplasia-pyometra complex.

Benesch and Power (1930) considered endometritis and pyometra in the bitch to be "diseases of city life". They observed that the majority of their cases were city animals leading a predominantly indoor life with only occasional exercise on a leash. Though their institute drew its patients almost equally from city and country, the incidence of the disease was lower in country bitches. These latter animals were allowed considerable freedom of movement and presumably

bred frequently. In the present survey, no comparison can be drawn between country and city dogs as the great majority of patients admitted to the hospital are from an urban area. It is significant that no report has been made of an analogous condition in wild carnivores and it would appear that the cystic hyperplasia-pyometra complex is a disease of domestication.

It is impossible to assess with any accuracy the significance, if any, of the preponderance of certain breeds in the series but it probably reflects the popularity of the various breeds in the area. Mongrel terriers and collies are the commonest and the cheapest pups displayed for sale in local pet shops. Small pure-bred terriers and Cocker Spaniels are popular household pets. Bull terriers and Alsatians are frequently used as watch dogs. A high incidence of the disease has been noted in the Bull terrier but this may be associated with the frequency with which this breed undergoes more than two cycles per year. The inclusion of the less common varieties of dogs in the series indicates that most, if not all, breeds are susceptible to the disease.

Another significant factor in the development of the various forms of the cystic hyperplasia-pyometra complex is revealed by the analysis of the distribution of the stage of oestrus cycle of the affected animals. The bitches included in Group I were in all stages of the cycle when examination was carried out. If the assumption is made

that cystic glandular hyperplasia of the endometrium is restricted to the period of ovarian activity one would expect to find the lesion during pro-oestrus, oestrus and metoestrus. However, the lesion is also present during anoestrus when ovarian activity is in abeyance. The presence of cystic glandular hyperplasia during anoestrus could be interpreted as an indication of continued and pathological ovarian activity. This interpretation is not supported by examination of vaginal smears which present the normal picture of anoestrus. Furthermore, in the ten anoestrus animals, histological examination of the ovaries reveals primary follicles and remnants of corpora lutea but no evidence of any well-developed structure capable of hormone secretion. This histological picture is consistent with normal anoestrus and in no way resembles the ovarian morphology during the active phases of the cycle. It seems more probable that cystic glandular hyperplasia can persist throughout anoestrus in the absence of hormonal stimulation. Braden and Peterson (1953) have shown that, in the guinea pig, artificially induced cystic glandular hyperplasia can persist for many months after the end of oestrogen treatment. They showed that sustained ovarian dysfunction is unnecessary to maintain the cystic changes in the endometrium since many of their guinea pigs later went through normal cycles whilst the lesion was still present.

In the remaining groups inflammatory changes are present in the endometrium only during metoestrus. The stage of cycle of the

affected animals ranged from five to 85 days after oestrus. This agrees with the findings of Kostner (1942) and Kammermann-Luscher (1952) though the former considers the disease is rare after the 70th day of metoestrus. The fact that inflammatory changes in the endometrium of the bitch appear to be restricted to the luteal phase of the cycle may be compared with the findings of Rowson, Lamming and Fry (1953) in the bovine. These workers showed that both exogenous and endogenous progesterone promoted conditions in the cow's uterus conducive to bacterial growth. Conversely, oestrogens rendered the bovine uterus resistant to infection. In the present study of the canine cystic hyperplasia-pyometra complex, all but one of the cases showing inflammatory lesions possessed morphologically functional corpora lutea in their ovaries. The association of corpora lutea and endometritis in the bitch was first noted by Devita (1939) in a small series of cases but he attached no great significance to this finding. Netzel (1935) had previously failed to mention the occurrence of corpora lutea in the ovaries of cases of the cystic hyperplasia-pyometra complex. He had produced a mild endometrial hyperplasia with oestrone in ovariectomised bitches. In a search for a possible source of oestrogen in natural cases, he observed that the ovaries of some of his cases contained follicular cysts. This, he assumed was a general characteristic of the disease and deduced that the various forms of the disease were different manifestations of hyperoestrinism. In the

present survey, follicular cysts were observed in 19 of the 100 cases examined but in only three of these were there no co-existent corpora lutea.

Bloom (1954) subscribed to the hypothesis advanced by Retzel but recognised that corpora lutea are frequently associated with the disease. He suggested the following explanations of this inconsistency:-

(1) the hyperplastic endometrium may be refractory to progesterone;

(2) the corpora lutea may be non-functional;

(3) the production of oestrogen may be so great that progesterone exerts no balancing effect.

All three possibilities may be refuted by the observations made in the present study. Histological examination of the ovaries reveals corpora lutea which appear active in all cases of endometritis. The secretory changes present in these uteri are identical to those produced by progesterone. The third possibility seems unlikely because signs of hyperoestrinism are absent. There is no nymphomania or anaemia suggestive of high oestrogen levels. The vaginal cytology is not characteristic of oestrogen stimulation. It seems probable that progesterone is the hormone determining the initiation and, possibly, the extent of the inflammatory reaction. This conclusion would explain the regression of inflammatory changes which follows ovariectomy in clinical cases.

The symptoms encountered in the clinical cases ranged from vulval discharge in otherwise normal dogs to abdominal distension, anorexia, emesis and coma. Cystic glandular hyperplasia of the endometrium uncomplicated by inflammatory changes is not usually clinically obvious and does not exhibit the haematological and biochemical changes which are readily detectable when inflammation has supervened. Haematological changes varied with the severity of the illness but neutrophilia was only slight in Group II. In Groups III and IV, neutrophilia was constant and often very marked. Haematological differentiation between the last two groups is in no way definite but certain differences may be observed. In the acute cases, "shift to the left" was always marked whereas in the chronic cases the number of immature cells was never great. In the chronic cases, it was frequently possible to observe abnormal mononuclear leucocytes; these so-called "star cells" are often found in other chronic diseases in dogs.

From the results of clinical examination and ancillary diagnostic aids, it is possible to classify affected animals according to severity of illness and according to the degree of retention of pus. In general, severity of illness is proportional to the degree of occlusion of the cervix hence it has become the practice to describe the condition as "open" or "closed" pyometra. This follows the original classification of Möller-Sörensen (1929) who divided the condition into pyometra and chronic catarrhal endometritis. It is known however that spontaneous cervical relaxation may occur in closed cases and, conversely,

closure may occur in previously discharging cases. In the present study, both open and closed cases appear in the same groups because they show similar histopathological lesions irrespective of the patency of the cervix. In the early studies of the disease, (Pronoh 1906 and Richter 1930), fluid retention was presumed to be due to inflammatory changes in the cervix. Bland (1929), discussing pyometra in women, listed as possible causes several conditions producing partial or complete closure of the cervical canal. He stressed the frequent association of the disease with fibrosis and tumours of the cervix. In the present study of 100 bitches, complete closure of the cervix was observed in only 15 cases though in many others, the patency of the canal was sufficient to allow drainage of only a fraction of the uterine fluid. In no instance was occlusion due to blockage by tumour of the cervical wall. Fibrosis of the cervix was recorded only in a few chronic cases. In most of the cases showing marked fluid retention, the ovaries contained corpora lutea which appeared morphologically functional even though the stage of cycle indicated that they should be regressing. Since spontaneous opening or closing of the cervix may occur during the course of the canine cystic hyperplasia-pyometra complex it would appear that the cervical occlusion is of a functional and not of an anatomical nature in the majority of cases. That it may be under the control of progesterone is suggested by the fact that occlusion of the cervical canal is a normal occurrence during metoestrus

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in the bitch.

The histological classification used in this study is not intended to be rigid. It is possible to trace a gradation in histopathological changes from the uncomplicated cystic glandular hyperplasia of the endometrium through acute endometritis to the plasma cell type and thence to chronic endometritis. While the changes may occur in this order in the natural disease, it is impossible on the present evidence to be dogmatic on this point and this subdivision of cases has been used to illustrate the histopathological relationships of these groups. The experimental evidence presented in the second part of the thesis supports the view that such a sequence does in fact occur and that endometrial hyperplasia can be a precursor of pyometra.

Bacteria were isolated from the uterine fluid in 82% of the clinical cases. There was considerable variation in the type of organism found and no organism was specific for any one type of endometritis. It is impossible to assess the importance of bacteria in the condition but it is significant that the organisms isolated are not commensals but are known pathogens. Whether they are secondary invaders as Kostner (1942) suggests or they are responsible for the inflammatory changes in uteri predisposed to infection, as Rieck (1956) suggests, cannot be answered without resorting to experiment.

From the findings of this survey, it can be concluded that the cystic hyperplasia-pyometra complex is a disease of the older, nulliparous

bitch which may become clinically manifest as an endometritis during the luteal phase of the oestrus cycle. Analysis of the factors involved in the development of the disease suggest that it is of hormonal origin; probably associated with ovarian dysfunction.

Section 2.

Experimental reproduction of the Cystic Hyperplasia-Pyometra Complex in the bitch.

Introduction.

Once the various manifestations of the cystic hyperplasia-pyometra complex had been studied in detail, it became necessary to attempt its artificial reproduction to obtain further information on the pathogenesis of the condition. Because experimental infection of the uterus with bacteria from natural cases has failed to reproduce the disease (Benesch and Farmer, 1930, Leshouyries and Bertholon, 1935, Tounlagen 1938 and 1952, and Kostner, 1942), no experiments of this type were attempted.

Cystic glandular hyperplasia of the endometrium is a common sequel to oestrogen treatment in the mammalian female. The lesion has been reported following a course of oestrogen injections in guinea pigs and rats (Wolfe, Campbell and Burch 1932), women (Kaufman 1934), mice (Parks 1935), monkeys (Zuckerman and Morse 1935), rabbits (Lacassagne 1935), ground squirrels (Wells and Overholser 1940) and golden hamsters (Bacon and Kirkman 1949). It would seem reasonable to expect that the lesions in the dog might be produced by a similar method. Retzel (1935), using large doses of oestrone, produced a mild form of cystic hyperplasia in normal and ovariectomised bitches but the lesions showed only slight resemblance to those observed in the natural disease. He inferred

from these experiments that cystic glandular hyperplasia of the endometrium in the bitches is the result of prolonged hyperoestrinism. Similar results were obtained by Devita (1952) and by Teunissen (1952) but because of the scant resemblence to the natural condition, neither worker was able to reach Hetzel's (1935) conclusion. Though Hetzel's hypothesis would explain the development of uncomplicated cystic hyperplasia, it does not explain why inflammatory lesions are observed only during the luteal phase of the cycle. Teunissen (1952) has shown that some degree of inflammatory reaction may be induced in the endometrium of bitches under the influence of progesterone.

The experiments in the following section were carried out on the assumption that the pathogenesis of the cystic hyperplasia-pyometra complex is intimately related to the ovarian hormones. The experiments are divided into three groups:-

Group A:- to observe the effects of duration of treatment and of dose of oestrogens on the endometrium.

Group B:- to observe the effects of duration of treatment and of dose of progesterone on the endometrium. This was done with progesterone alone and following oestrogen sensitisation.

Group C:- to observe the effect of repeated alternation of oestrogen and progesterone. This is an attempt to simulate a number of cycles since it has been shown in the previous section that a number of cycles must occur before the appearance of the natural disease.

Materials and Methods.

Bitches of nine to 15 months old were bought from a local pet shop. Animals of this age were chosen because they were not within the age range of the naturally occurring disease and were therefore unlikely to have spontaneous lesions. The animals were predominantly collies, weighing from eight to 14 Kg. but a few mongrel terriers were used. They were submitted to bilateral ovariectomy and at the same time, opportunity was taken to observe the gross state of the uterus. Where pregnancy was present or where the uterus showed evidence of previous pregnancy, the animal was discarded from these experiments and used for other purposes. An interval of 28 days elapsed between ovariectomy and the start of an experiment. As well as permitting complete recovery from operative procedures, this interval allowed time for inactivation and excretion of endogenous ovarian hormones and for regression of the cyclic changes of the endometrium.

2.5 ml. of blood was withdrawn from each animal every third day for haematological examination both before and during the experiment. Every seventh day plasma urea, serum bilirubin, calcium and inorganic phosphate levels were estimated. Vaginal smears were examined daily.

At the end of each experiment, hysterectomy was performed and the uterus was submitted to the histological examinations described in the previous section.

The progesterone used in these experiments was an ethyl oleate suspension supplied by Organon Laboratories, Ltd. The oestrogens were stilboestrol dipropionate in arachis oil (B.W. and Co.) and oestradiol monobenzoate in ethyl oleate (Organon).

Full details of the dose and duration of treatment are given in each experiment.

Group A.

The Effect of Oestrogens on the Endometrium of the Overiectomised Bitch.

Experiment 1.

Two overiectomised bitches, cases D1 and D2 received daily intramuscular injections of 10 mg. of stilboestrol dipropionate in oil for 10 days. They were hysterectomised on the day following the last injection.

By the third day, the vulvae of both animals were considerably swollen, congested and oedematous. In case D1, a slight vulval discharge became apparent on the fifth day. The discharge was of a clear mucoid nature and persisted until hysterectomy without becoming blood-stained as in normal pro-oestrus. In case D2, discharge did not appear until the ninth day and was sanguinous from the start. There was no observable hypertrophy of the mammary glands in either animal. Both were attractive to male dogs during the latter half of the period of treatment but resulted any attempt on the part of the males to mate.

Haematology.

A leucocytosis commenced on the third day of treatment and the white cell counts rose gradually to reach between 25,000 and 30,000 per c. mm. on the 10th day. The leucocytosis was due entirely to an absolute increase in the number of neutrophils of which up to 5% were

of the non-lobulated variety. There were no significant changes in the absolute numbers of other leucocytes. Slight falls in the erythrocyte counts and haemoglobin concentrations were noted over the last few days of treatment. In case B2, the erythrocyte sedimentation rate (E.S.R.) was gradually accelerated to reach a rate of 32 mm. per hour on the 10th day. In case B1, a slight increase in the E.S.R. was noted on the final day.

Biochemistry.

The plasma urea and serum bilirubin levels remained constant throughout the experiment in both animals. In case B2, the serum calcium level rose slightly and the inorganic phosphate level fell proportionately towards the end of the period. Serum calcium and inorganic phosphate concentrations remained within normal limits in case B1.

Vaginal Cytology.

Initially, the smears were similar to those of ex-oestrus but changes in cell type soon became evident. The first cornified cells were observed on the third day in both animals and by the 10th day, over 80% of the cells had undergone cornification. The proportion of exudated cornified cells in the vaginal smears during the latter part of the experiment was greater than that observed in the normal oestrus animal. Though erythrocytes were present in the smears at an early stage in the treatment, they never attained the vast numbers normally seen in pro-oestrus and oestrus smears.

Normal Anatomy.

During the period of treatment, the uterine horns had increased from less than 0.5 cm. to over 1 cm. in diameter. They were rounded and tense (Fig.44) and the endometrial surface was marked by a number of longitudinal ridges and was pale pink. The uterine lumen did not contain mucin or blood. Bacteriological swabs taken from the endometrial surface failed to yield organisms on cultural examination.

Histology.

The endometrium is greatly swollen and the uterine lumen is reduced to a narrow H-shaped slit (Fig. 42). There is considerable oedema of all layers of the endometrium and the vessels are prominent and congested. This congestion is particularly evident in the superficial capillary bed which is usually difficult to detect. There is diffuse extravasation of red blood cells throughout the crypt layer. Small focal haemorrhages are present in other areas in case D1 but are not a prominent feature. There is no evidence of vascular damage to suggest any mechanism for the steroid haemorrhage. It is almost certain that the blood in the vaginal mucus is of uterine origin but no breaks in the surface epithelium of the endometrium can be found.

The superficial epithelium is composed of a single layer of cells which vary in height from cuboidal to low columnar in any one section (Fig. 43). The cells are broad and have a pale, homogeneous eosinophilic cytoplasm bounded by distinct nuclear membranes. The nuclei

vary in shape from round to oval. The long axes of the nuclei are generally parallel to the surface but some have become orientated at right angles to it. The deeply staining nuclei are comparatively large and are situated in the middle of the cells. Only occasional, isolated mitotic figures are visible in any one cross section.

There appears to be an increase in the number of crypts or glandular orifices but most of them are short and the crypt zone is of normal breadth. They are bottle-shaped with comparatively long, narrow necks opening into wider, rounded bodies. The cells near the mouth of the crypt at the junction with the surface epithelium are tall and closely packed. The nuclei are long, slender and hyperchromatic with occasional mitotic figures. These cells gradually decrease in height down the neck of the crypt and the cells lining the body are of a low columnar type. The nuclei of the latter are comparatively large, oval in shape and stain less intensely. There is no evidence of secretion in the lumina of the crypts.

The tubular zone is very cellular and is the broadest layer of the endometrium. There are relatively few glandular tubules in the zone. They are mainly straight but a few show evidence of slight tortuosity at their basal ends. The lumina are narrow and do not contain secretion. They are lined by a single layer of medium-sized columnar cells. These cells have a homogeneous eosinophilic cytoplasm and relatively large oval nuclei situated near the basement membrane.

Only rarely are mitotic figures present in the tubular epithelium.

In the basalis zone, there is a small but definite increase in the number of glandular lumina per field. The units tend to occur in small groups separated from neighbouring groups by a stroma which is wider than that which separates individual members of a group. There is evidence of coiling in some areas and occasional branching of glands is present. The glandular lumina are, in general, narrow but a few isolated units are dilated to a moderate degree. Cystic dilation of glands is not present in any section. None of the units contain secretion. The glands are lined by a cuboidal or low columnar epithelium which is lowest in those which are dilated. Mitotic figures in the epithelial cells are rare.

Traces of cytoplasmic glycogen are present in the superficial epithelial cells, particularly, close to the free border. Glycogen is not apparent in the epithelium at other levels of the endometrium. Granules, having the staining characteristics of acid muco-polysaccharide are present in the epithelium of all zones of the endometrium. These granules are present only in the supranuclear cytoplasm and are most numerous near the free edge of the cells. They are relatively scanty in the superficialand crypt epithelium but gradually increase in number down the tubules and are most numerous in the basal glands. There is no submucosal material in the epithelium at any level of the endometrium.

No significant changes referable to oestrogen stimulation can be detected in the myometrium of either case. The inner layer of muscle

appears normal compressed but this is probably due to endometrial tension. The vessels of the gravidus vasculare appear hypertrophied and coiled. The connective tissue of the stratum vasculare is negligibly edematous. The penetration is thin and tense unlike the normal ruminated arrangement.

Experiment 2.

Two ovariectomized kittens, cases D3 and D4, each received daily intramuscular injections of 10 mg. of stilbocentrol dipropionate in oil. The intended duration of treatment was 30 days but D3 died on the 21st day and D4 died on the 25th day, having received a total of 200 mg., and 250 mg. of stilbocentrol respectively.

Both animals appeared normal until the day prior to death when they became reluctant to exercise and showed loss of appetite. The illness then progressed rapidly to complete stasis and finally, coma. Severe ascitis was observed in the last few hours before death. Conjunctival bleeding and petechiation of the conjunctival and oral mucosa were observed in both animals. D4 passed haemorrhagic faeces several times in the terminal stages. Both had previously exhibited profuse, blood-stained vaginal discharge between the ninth and 12th days of treatment but this had disappeared completely before toxic symptoms became evident. Coitus had shown interest in both animals but the

biscuits would not permit eating.

Haematology.

In B5, the total white count rose gradually from the third day to reach 42,000 per c. mm. on the day of death. In B6, the white cell count was 63,000 per c. mm. on the 8th day of treatment and had fallen to 4,000 per c. mm. just before death two days later. In both, the leucocytosis was due to neutrophilia. The E.S.R. was accelerated to over 40 mm. per hour in the later stages of treatment. The erythrocyte count in both animals fell by almost two million cells per c. mm. during the experiment and corresponding decreases in haemoglobin concentrations were recorded.

Biochemistry.

Plasma urea and serum bilirubin levels remained constant throughout the experiment. Both animals showed a slight fall in serum inorganic phosphate and a corresponding rise in serum calcium during the middle stages of the experiment but these rapidly returned to normal.

Vaginal Cytology.

The first cornified epithelial cells were observed in smears taken on the third day of treatment and from the 12th day onwards, about 100% of the cells were of the cornified variety. In B3, a few red cells were seen in the smears taken on the sixth and seventh days but at no other time. In B4, large numbers of red cells appeared on the sixth day and persisted until the 12th day before gradually

Screening.

Morbid Anatomy.

Autopsy findings were almost identical in both animals. There was a slight subcutaneous oedema in the mandibular space and in the ventral cervical and anterior thoracic region. Subcutaneous haemorrhages were present at points liable to traumatic injury such as the elbows and hocks. Intramuscular haemorrhages of small volume were present in relation to the last few injections of stilboestrol. Numerous petechiae were evident on the oral mucosae and fauces. Ecchymotic haemorrhages were present in the gastric and intestinal mucosae of both animals. In D_1, there was a considerable volume of unchanged blood in the colon but no definite site of origin could be traced. Small irregular haemorrhages were present under the abdominal and thoracic serosae particularly in the omentum and in thymic region. There were numerous petechiae and slightly larger haemorrhages scattered under the epicardium and concentrated around the coronary grooves. The heart was pale and flabby with no evidence of haemorrhage into the myocardium. Sub-endocardial haemorrhage was prominent over the papillary muscles, on the atrio-ventricular valves and in the auricular appendages. The lungs were moderately oedematous and the bronchi contained some frothy blood-stained fluid. A few sub-pleural haemorrhages were present on the anterior lobes of the lungs. The liver was pale and fatty in appearance. Sub-periosteal haemorrhages were present in the limb bones.

The femoral marrow was oedematous and very congested. The uteri showed a greater degree of hypertrophy both in width and length than those in experiment 1. The lumina did not contain fluid and were bacteriologically sterile on cultural examination.

Histology.

Microscopic haemorrhages are present in lungs, spleen, kidneys, thymus and adrenal glands in addition to the organs mentioned at autopsy. Slight centrilobular fatty change is present in the liver of both animals. Examination of sections of the femoral marrow reveals marked changes in composition. In both cases, there is marked hyperplasia of the myeloid elements with a predominance of immature forms. Erythroid elements form small islets scattered among the masses of myeloid tissue. In D_4, there are what appear to be small foci of hypoplasia. These are small areas composed of a thin oedematous stroma containing only a few early erythroid cells but no myeloid elements.

Uterus.

Histological examination of the endometrium reveals few differences from the reaction observed in experiment 1. The endometrium is swollen and oedematous (Fig. 44). Haemorrhage in the crypt zone is slight and focal in nature. Numerous haemosiderin-containing macrophages are present in all strata of the endometrium suggesting that the haemorrhage has been actively reabsorbed.

The superficial epithelium is composed of moderately tall columnar

cells with rare basal, oval nuclei orientated at right angles to the basement membrane (Fig. 45). There is a slightly increased mitotic rate. The cytoplasm is less distinctly homogeneous and the cells are more closely packed together.

The crypts are no more numerous than in Experiment 1 but the breadth of the crypt zone has increased. The crypts are bottle-shaped but the necks are less constricted and the difference between the epithelium of the neck and the body is less obvious.

There is no increase in the number of tubules but they are more tortuous. The lining epithelium is lower than that of the surface and the lumina are devoid of secretion. The ovoid nuclei are orientated with their long axes perpendicular to the lumina.

In the basilla zone, the number of mitoses per field is greater than that observed in Experiment 1. The zone is broader, the number of cells in each group is greater and there is evidence of coiling of the glands. The number of glandular branches is increased. In most glands, the lining epithelium is composed of medium-height columnar cells with ovoid nuclei situated close to the basement membrane. The long axes of the nuclei are at right angles to the basement membrane. The average diameter of the cells is greater than that observed in Experiment 1 and the number showing abnormal division is also greater. These distant glands are lined by a low cuboidal epithelium with relatively large, deeply staining nuclei in a scanty cytoplasm. Small globules of

secretion are present in some cells but this is not a persistent feature.

Glycogen granules are present in the superficial epithelium and in occasional cells in the crypts. Acid muco-polysaccharide is present in the supranuclear cytoplasm of the epithelial cells in all levels of the endometrium. These granules are most numerous in the basal glands and least in the superficial epithelium. The secretion observed in occasional tubules and acini contains acid muco-polysaccharide.

Experiment 3.

Two ovariectomised cattle bitches, cases 85 and 86, each received daily intramuscular injections of 3.25 mg. of oestradiol monobenzoate dissolved in ethylene. This dose of oestradiol monobenzoate was chosen because it has an oestrogenic potency equivalent to that of 10 mg. of stilboestrol dipropionate. 86 died on the 19th day and 85 on the 27th day after receiving total doses of 60 mg. and 86.63 mg. of oestradiol monobenzoate respectively.

Both animals after a short illness culminating in complete atonia and then ataxia similar to that observed in the animals receiving an equivalent dose of stilboestrol dipropionate. Haemorrhage from the body orifices did not occur in either animal.

Haematology.

Both animals showed the progressive neutrophilia and the gradual

anaemia characteristic of oestrogen treatment.

Biochemistry.

No relevant changes were observed in the plasma urea, serum bilirubin, calcium and inorganic phosphate levels.

Vaginal Cytology.

The cell changes in the vaginal smears are similar to those recorded in Experiment 2.

Morbid Anatomy.

The autopsy picture in both animals was characterised by a purpura of the type described in the previous experiment.

Histology.

Examination of the endometrium reveals a similar picture to that observed in the animals receiving an equivalent dose of stilboestrol dipropionate. There is marked oedema of the endometrium with focal haemorrhage and slight dilation of some of the basal glands.

Experiment 4.

Two ovariectomised collie bitches, cases D7 and D8, each received daily intramuscular injections of 5 mg. of stilboestrol dipropionate in oil for 10 days. They were hysterectomised on the day after the last injection.

On the fourth day, both animals showed evidence of vulval oedema and attraction for males. Vulval discharge became apparent on the

ninth day and quickly became blood stained. Both animals eat and healthy throughout the experiment.

Hematology.

In #7, metacephilia commenced on the third day and by the 10th day, the total white count was in excess of 20,000 cells per c. mm. The leucocyte counts of #8 remained within normal limits until the last day when a count of 19,500 cells per c. mm. was recorded. In neither case were significant changes observed in the hemoglobin concentrations or erythrocyte counts. In both animals, the N.P.N. rose gradually throughout the experiment.

Biochemistry.

No changes were observed in the plasma urea and serum bilirubin levels. Serum calcium and inorganic phosphate levels rose and fell slightly during the experiment without showing a significant trend of change.

Vaginal Cytology.

The differential cell counts followed the basic pattern of gradual epithelial cornification described in previous experiments.

Morbid Anatomy.

The uterine horns exhibited a slight degree of hypertrophy. The lumen did not contain fluid.

Histology.

The endometrium in section and sedimentary with diffuse hemorrhage

throughout the crypt layer. The epithelium at all levels of the endometrium is similar to that observed in animals treated with larger doses for a similar period. The crypt zone is narrower than in the latter but a similar increase in the number of glandular buds in the basal zone is present. The mitotic rate throughout is low. Acid muco-polysaccharide granules are present in the epithelial cells of all layers and traces of glycogen are present in the surface epithelium.

Experiment 5.

Three ovariohysterectomised bitches, ones D9, D10 and D11 each received daily intramuscular injections of 5 mg. of stilboestrol dipropionate in oil. All stilbinbed vulval oedema from the third or fourth day onwards. In the later stages, the grossly enlarged vulvae were frequently ulcerated and scarred by injury. Vulval discharge became apparent on the sixth or seventh day of treatment and disappeared by the 18th day. During the early stages of the experiment, none of the bitches would permit mating though males were physiological in their attention. During the middle stages, they would have mated but were prevented from doing so. Later the bitches showed nymphomanic tendencies but the attraction for males appeared to have worn off despite the obvious postural invitation to mate on the part of the

hrs. D9 died on the 30th day, D11 on the 33rd day and D10 on the 36th day of treatment. Illness was of the same type in each animal. Anorexia was apparent on the day prior to death and at autopsy on the following morning muscular incoordination was noted. The illness then progressed rapidly to complete paralysis, coma and death within a few hours. Hemorrhages from the lips, ears and rectum occurred in D10 in the last few hours before death.

Hematology.

Progressive neutrophilia started on the third day of treatment. On the day of death, D9 had a total white cell count of 27,000 per c. mm. In the other two animals, neutrophilia was sustained until the 30th day when a sharp fall in the leucocyte count occurred. In under 48 hours, leucocyte counts fell from over 35,000 per c. mm. to under 5,000 per c. mm. In this change there was a decrease in the absolute numbers of all leucocytes though the decrease was largely in the neutrophil count. A reversal of the normal lymphocyte-neutrophil ratio occurred. All these animals had a marked anemia before death. Over the period of treatment, the erythrocyte counts had gradually fallen by over 2,000,000 cells per c. mm. with corresponding decreases in haemoglobin concentration. Erythrocyte sedimentation rates were gradually accelerated in the later stages of treatment.

Biochemistry.

Significant changes were not observed in the serum concentrations

of bilirubin, calcium, inorganic phosphate and alkaline phosphatase or in the plasma urea levels.

Vaginal Cytology.

The differential cell counts of the vaginal smears followed the same pattern as in previous experiments. In the latter half of the period, all the cells in the smears were of the cornified variety.

Morbid Anatomy.

All these animals showed evidence of purpura with numerous small haemorrhages on the serosae and mucosae of the body. Gross haemorrhage had occurred in the stomach of D11. In D9, the femoral marrow was markedly congested but in the other two animals, it was oedematous and pale in colour. The uterine horns were thickened and coiled but did not contain fluid. Cultural examination of swabs taken from the uterine lumina failed to yield organisms.

Histology.

Examination of the various organs reveals the pattern of widespread petechiation which is the most prominent feature of stilboestrol poisoning in the dog. In D9, the marrow is congested and shows marked myeloid hyperplasia.

In the other two animals, there is hypoplasia of both erythroid and myeloid elements. The marrow is composed of a thin, oedematous connective tissue stroma in which are scattered foci consisting of a few erythroid and myeloid cells.

Uterus.

The endometrium is less markedly oedematous than has been observed with shorter periods of treatment (Fig. 46). There is no haemorrhage in D10 and D11 but a few small foci are present in D9. Haemosiderin-containing macrophages are present in all sections but are not numerous. A few of these macrophages have penetrated the basement membrane of the superficial epithelium. The vessels of the endometrium are prominent but are not dilated though their walls appear to have hypertrophied. The superficial capillary bed is dilated. The reticulin network of the crypt zone is decreased in thickness and has been replaced by fine collagen fibres. The endometrial stroma is composed largely of thickened collagen fibres and is relatively acellular (Fig. 47). Inflammatory cells are not present in any of the sections examined.

The superficial epithelium is composed of tall columnar cells with pale homogeneous cytoplasm. The nuclei are elongated and closely packed with their long axes at right angles to the basement membrane. Mitotic figures are rare.

The crypt zone is wider than that observed in the previous experiments but there is no evidence of further increase in the number of crypts. The crypt necks are dilated and merge imperceptibly into the bodies. The cells are slightly taller than those of the surface. The nuclei are less closely packed and are irregularly orientated but remain close to the basement membrane. The cytoplasm is paler and contains

numerous small vacuoles. Mitotic figures are frequent.

There is no obvious increase in the number of tubules but they have become markedly tortuous so that a number of tangential and oblique sections of each tubule can be seen. The diameter of the tubules is generally greater than has previously been observed and some are dilated throughout their length. The epithelium varies with the diameter of the tubules but, in most, it is composed of a single layer of cuboidal-cued columnar cells. In dilated tubules, the cells are lower and the cytoplasm of the epithelium is homogeneous.

There is no greater increase in the number of glands in the breast than has been previously observed. The acini vary in size but are all larger than those in earlier experiments. Some acini are dilated beyond average diameter and may be considered cystic though not comparable in size to those of the natural condition. The lining epithelium varies from columnar in the average glands to cuboidal in those which are dilated. The mitotic rate is low and dilation does not appear to be directly related to proliferative changes in the epithelium. Lactational glands contain secretion.

Glycogen granules are present only in a few epithelial cells in the crypts. Acid mucopolysaccharide is present in the crypt, tubular and basal glandular epithelium, particularly in the latter, but is not abundant.

The myometrium is hypertrophied and the connective tissue elements

cle were prominent. The stroma vascular is not edematous but the vessels are very tortuous and their walls are hypertrophied. The vessels traversing the inner myometrium are abnormally prominent.

Experiment 4.

Two overlactated mongrel terrier bitches, cases D12 and D13 received daily intramuscular injection of 25 mg. of stilboestrol dipropionate in oil. D12 died on the eleventh day after treatment and D13 on the 15th day after having received 250 mg. and 300 mg. of stilboestrol dipropionate respectively. Both animals died within a few hours of running convulsions prior to which no symptoms were observed. Death was preceded by severe vomiting and rapid wheat of coma. The kidneys were characterised by gradual decrease in the volume of the pulse and increase in rate. There was increased rate and depth of breathing which finally became irregular and panting in type. The oral mucosa and conjunctivae were pale. Though all symptoms were indicative of haemorrhage, there was no evidence of it from any of the body orifices.

Haematology.

In D12, the total white cell count rose from 12,000 per c. mm. on the third day of treatment to 45,000 on the seventh day and remained at this order until the animal died. Initially, the neutrophilia was characterised by a high percentage of immature cells but in the later

otogen, only mature cells were apparent. Insignificant depressions were noted in the erythrocyte counts and hemoglobin concentrations. In #13, there was only slight stimulation of the hemopoietic tissues and the leucocyte count did not rise above 15,000 cells per c. mm. Both animals had erythrocyte sedimentation rates of over 40 mm. per hour during the last day of life.

Biochemistry.

No relevant changes occurred in the plasma urea and serum bilirubin concentrations. In both cases the serum calcium level rose and the inorganic phosphate level fell.

Vaginal Cytology.

Though cornified cells did not appear until the third day of treatment, the rate of transformation was more rapid than that observed at lower dosage rates. By the sixth day, over 50 % of the cells were cornified. Cornification was present not only in the large epithelial cells but in the small basophilic which do not normally undergo transformation.

Gross Anatomy.

In both animals, the immediate cause of death was internal hemorrhage. In #12, the stomach contained a considerable volume of blood but no one site of origin could be traced because the entire fundic mucosa appeared hemorrhagic. In #13, similar hemorrhage had occurred in the duodenum. Lesions were not observed elsewhere in either animal. The uteri were hypertrophied to an extent greater than that observed at

lower dosage levels. Fluid was not present in the uterine lumen.

Histology.

In D12, there is considerable mucosal hemorrhage in the gastric fundus and similar hemorrhage is present in the duodenum of D15. There is no microscopic evidence of the widespread petechiation which characterizes the toxic action of coccivogen at lower dosage rates.

The hypertrophy of the uterus appears to be due largely to gross edema of the endometrium. Extravasation of blood into the crypt zone is more marked than in any other experiment hitherto described. Despite the severity of hemorrhage it is not possible to detect vascular damage or rupture of the superficial epithelium. The superficial epithelium is composed of tall columnar cells which are closely packed together. The cytoplasm of these cells is a pale homogeneous, eosinophilic colour. The nuclei are elongated and rather deeply staining. The mitotic rate is low.

There is no increase in the number of crypts which are lined by an epithelium which is only slightly lower than that of the surface. The crypt necks are constricted and the bodies of the crypts are dilated.

The tubular zone is extremely broad but contains only a few widely separated tubules. These tubules are straight with comparatively narrow lumina and are lined by a tall columnar epithelium.
The basal glands show no definite increase in number but there are occasional branches and early coiling is evident in some areas. The

canal are lined by an epithelium which is considerably lower than that of the tubules. Only occasional glandular lumina are dilated.

Discussion and Conclusion.

The dosage levels of oestrogen chosen for the first six experiments were within the toxic range hence the duration of treatment was limited by the survival time of the animals used. The toxic action of oestrogens in the dog appear to be mainly directed against the haemopoetic tissues causing an initial stimulation followed by destruction of all marrow elements. Death in all cases was preceded by the onset of symptoms of thrombocytopenic purpura and, in some cases, by gross internal haemorrhage. At autopsy there is little evidence of the hepatic damage which is so characteristic of oestrogen poisoning in the cat (Dow, 1938).

Ovariectomised bitches treated with large doses of oestrogen for periods of up to 30 days failed to develop lesions comparable to naturally occurring cystic glandular hyperplasia of the endometrium. The changes produced in every case are similar to those observed in pro-oestrus or oestrus or are merely an exaggeration of these. The intense uterine oedema and the extravasation of blood into the crypt zone which are characteristic of oestrus are reproduced. It would appear from the observations made that the haemorrhage of oestrus is not a continuous process but occurs only in the early stages of oestrogenic stimulation.

re-absorption of the hemorrhage by macrophages is prominent in all layers of the endometrium. This endometrial hemorrhage is in no way associated with the general purpura which precedes death but occurs before toxic changes are apparent in the peripheral blood.

As in normal oestrus, there is proliferation and hypertrophy of the superficial and glandular epithelium of all layers of the endometrium. It is of interest to note that secretory granules appear in the cytoplasm of the endometrial epithelium under the influence of oestrogen alone. Even if these animals had been under the influence of progesterone prior to ovariotomy, at least 28 days had elapsed before commencing oestrogen treatment. The basal glands show evidence of branching and of dilation, particularly in those animals undergoing more prolonged treatment. Such dilation of the basal glands is not a normal feature of oestrus in the bitch but the degree of dilation is in no way comparable to that observed in naturally occurring cystic glandular hyperplasia of the endometrium. The endometrium in oestrogen treated animals does not show the widespread and irregular dilation of the natural disease. In no animal is there evidence of intense proliferation of the superficial and crypt epithelium to produce a complex system of crypts and polyps such as is seen in the natural condition. Cellular infiltration of the endometrium is not present in any case.

The slight differences in reaction noted with increasing oestrogen

levels are not proportional to the daily dose administered. A slightly better correlation exists between the degree of dilatation and the duration of treatment but even this is limited. Both Swanson (1932) and Davis (1932) have treated bitches with large dosage ratios of oestradiol for periods up to 60 days but they found little tendency for the dilatation of the glands to progress. Swanson found that, as in the present experiments, oestrogenic equivalents of oestradiol and stilboestrol dipropionate produced similar results. The results of the present experiments and those of Swanson (1932) and of Davis (1932) are at variance with those of Hetzel (1935) who claimed that he had produced cystic glandular hyperplasia of the endometrium in ovariectomised bitches by similar methods. From Hetzel's photomicrographs, the difference appears to be of interpretation of the term cystic glandular hyperplasia rather than of experimental results.

Though the results obtained in the present experiments may be taken as evidence that the duration of treatment is insufficient, it is in excess of that required to produce cystic glandular hyperplasia of the endometrium in rodents (Wolfe et al 1932 and Lemmegre 1935) and similar to that required to cause these changes in guinea-pigs (Zuckerman and Home 1935). It is evident that some degree of glandular dilatation may be produced in the canine endometrium by oestrogen stimulation. The changes produced are insufficient to permit Hetzel's deduction that hyper-oestrinism alone is responsible

for all the pathological manifestations of the cystic hyperplasia-pyometra complex. In the normal, ovarian cycle of the bitch, each oestrogen phase is followed by a period of progesterone activity and then by a period of sexual inactivity. It seems probable that either or both of these stages are essential in the development of cystic glandular hyperplasia of the endometrium.

Group B.

The Effect of Progesterone on the Endometrium of the Ovariectomized Bitch.

Experiment 7.

Six ovariectomized bitches received daily intramuscular injections of 10 mg. of progesterone in oil for 10 days and were then hysterectomized. Dogs D14 and D15 received no treatment prior to the start of progesterone injections but the others were sensitized with oestrogens. Dogs D16 and D17 received 5 mg. and D18 and D19 received 10 mg. of stilboestrol dipropionate daily for 10 days. The last oestrogen and the first progesterone injections were administered on consecutive days. In the animals pre-treated with stilboestrol, the characteristic reactions were observed but there were no secondary sex changes referrable to progesterone activity.

Haematology.

In dogs D14 and D15 which received progesterone alone there were

no significant changes in the cytology of the peripheral blood. In the other four which were pre-treated with stilboestrol, the changes produced by stilboestrol continued during the progesterone treatment. The neutrophilia in each animal reached its peak between the third and the fifth days of progesterone treatment. In M9, anaemia and leucopenia were marked on the last day of treatment and she failed to survive hysterectomy.

Biochemistry.

Changes were not observed in the plasma urea or in the serum bilirubin, calcium, inorganic phosphate and alkaline phosphatase levels

Vaginal Cytology.

In the two animals receiving progesterone alone, there was a gradual increase in the number of small basophil epithelial cells during treatment. A few polymorphonuclear leucocytes were apparent on the first few days but had disappeared by the fifth day. In those which received stilboestrol prior to the progesterone injections, cornification reached its peak at the fourth or fifth day after the last stilboestrol injection and gradually regressed until the end of the experiment.

Normal Anatomy.

All the uteri were slightly enlarged and more vascular than normal. The increase in length and in breadth was greater in those animals which had been pretreated by stilboestrol.

Histology.

Cross-sections of the uteri reveal an increase in thickness of the endometrium with considerable reduction in the diameter of the uterine lumen (Fig. 48).

The superficial epithelium is composed of single layer of tall columnar cells with distinct cell membranes and smooth level surfaces (Fig. 49). The nuclei are large and vesicular. They are oval in shape and are situated in a regular line near the base of the cells. The nuclei are more crowded, longer and less regularly arranged in the oestrogen-sensitised animals. The cytoplasm of the cells is of a pale eosinophilic colour and is not homogeneous but is stippled with very small vacuoles. In the oestrogen-sensitised animals, occasional cells contain a single supranuclear vacuole of a round or more frequently crescentic shape. Such vacuolated cells tend to be broader than their neighbours and are raised slightly above the general level of the epithelium. Mitotic figures are rare and occur in only a few of the many cross-sections examined.

There is an increase in the number of crypts visible per cross-section in all cases. The crypt mouths are open and the lumina are wide. At the point of mergence of the crypt epithelium with the superficial epithelium there is considerable proliferation of the cells with numerous mitoses. Here the cells are slender with elongated hyperchromatic nuclei. These cells are parallel to those on either side

and do not possess any of the staining affinities of uro-epithelial cells which they resemble. The cells lining the bases of the crypts are taller than those of the surface epithelium. The nuclei are basal in position but are less regularly arranged so that at points they appear pseudo-stratified. They are large and vary from round to oval. Occasional mitotic figures are present and are situated on the luminal edge of the epithelial layer. The cytoplasm is paler than that of the surface epithelium and is more distinctly vacuolated. Some cells with excess vacuolation have the appearance of goblet cells with a broad surface narrowing towards the base.

The form of the crypts is the same in all animals but there does appear to be a slight increase in number in those receiving oestrogen treatment.

There is an increase in the number of lumina present in the tubular zone of the endometrium in all cases. The tubules are almost all straight until they enter the basal zone but a few tortuous tubules are visible in the oestrogen-conditioned zone. The lumina of the tubules are broader than normal and are round or cross-shaped. The tubules are lined by a single layer of tall columnar cells with polo, basal nuclei (Fig. 50). The nuclei are closely packed and are almost rectangular in outline. The abundant cytoplasm is finely granular and in many cells a large round supranuclear vacuole is present. Occasional cells also possess small subnuclear vacuoles. At irregular intervals, there are slender

cells with scanty cytoplasm and much taller, hyperchromatic nuclei compressed between the normal epithelial cells. Cells in mitosis are more numerous than in the more superficial layers of epithelium. These mitotic figures are always situated on the luminal edge of the epithelial layer. The mitotic rate bears no relation to the state of the endometrium prior to progesterone treatment.

In the basilla zone, there is a distinct increase in the number of glandular lumina associated with each tubule. Though branching of the tubules is evident, it would appear from the orientation and arrangement of the glands that much of the increase is due to coiling of the tubules. The cells lining the glands vary considerably in size but are generally smaller than those in the tubules. In some areas, the cells are low columnar and the cytoplasm is scanty whilst in others they are taller with abundant cytoplasm. The cytoplasm is more homogeneous than in the superficial layers and secretory vacuoles are less numerous. The mitotic rate is comparable to that of the tubular epithelium. The lumina of the basal glands are narrower than those of the tubules in those animals receiving progesterone alone and are of uniform diameter. In the oestrogen-sensitised animals, the lumina are generally larger and vary considerably in diameter though gross dilatation is not present. Histological techniques reveal the presence of acid muco-polysaccharide in the supranuclear cytoplasm of the epithelium of all layers. Secretory granules are most abundant in the crypt and tubular epithelium, and least numerous in the superficial epithelium.

There is a slight accumulation of glycogen in the cytoplasm of the surface epithelium particularly along the luminal edge. Secretion is not present in any of the glandular tissue.

In those cases which received stilbocontrol, there are small areas of hemorrhage in the stroma cells and hemosiderin-containing macrophages are scattered throughout all layers of the endometrium. In all cases, there is a mild edema of the endometrium with swelling of the collagen fibers. The number of stromal cells is increased and their nuclei are larger and more ovoid than those observed in the first series of experiments. Mitotic figures are not infrequent. There are no abscess or inflammatory cells in the endometrium.

The inner circular layer of myometrium appears compressed and the nuclei are large and slender. There is a variable degree of edema in the stratum vasculare.

Experiment 3.

Six ovariectomized bitches received daily intramuscular injections of 10 mg. of progesterone for 20 days and were then hysterectomized. Cases B20 and B21 received no treatment prior to the start of progesterone injections but the others were sensitized with oestrogens. Cases B22 and B23 each received 5 mg. and B24, and B25 each received 10 mg. of stilboestrol dipropionate daily for 10 days. The last oestrogen and the first progesterone injections were administered on consecutive days.

D12 to D25 showed the characteristic reactions to oestrogen administration but these rapidly disappeared after progesterone treatment was started. There were no observable secondary sex changes which could be traced to progesterone activity. Abscesses followed almost every progesterone injection in D24 and it was destroyed at the end of the experiment.

Haematology.

No significant haematological changes were observed in D20 and D21 which received progesterone alone. In those animals which were pre-treated with stilboestrol, the reactions produced by oestrogen activity continued into the progesterone period for several days before returning to normal. The neutrophilia reached its peak between the third and seventh day of progesterone treatment. In D24, a moderate neutrophilia continued until the end of the experiment but this can probably be accounted for by the abscesses produced by progesterone activity.

Biochemistry.

There were no significant changes in the serum concentrations of bilirubin, calcium, inorganic phosphate and alkaline phosphatase during any part of the experiment. The plasma urea level rose during the period of experiment in D23 but this was traced to a mild leptospiral nephritis.

Vaginal Cytology.

In cows D20 and D21 which received progesterone alone, there was

a gradual increase in the number of small basophil epithelial cells during the course of treatment. Neutrophils were evident in the smears of both in small numbers during the first 10 days but disappeared after that. In the four ovariectomized-treated animals, the cornification reached its peak at about the fourth day of progesterone treatment, regressed gradually until the 10th day and thereafter cornified cells were rarely observed. In Case 329 erythrocytes were present in the smears up to the 10th day of progesterone treatment.

Normal Animal.

All the uteri showed approximately the same degree of hypertrophy which was slightly greater than that observed in experiment 7. It was noted at hysterectomy that the cervix was tightly closed. A small amount of mucoid fluid was present in the lumen of each uterus. The uterine fluid was sterile in each case.

Histology.

The superficial epithelium is considerably taller than that observed in the previous experiment. The nuclei have moved away from the basement membrane and now occupy the inner third of the cells. They are closely packed and the variation in position gives the epithelium a pseudo-stratified appearance. The nuclei are large, oval and pale staining. The cytoplasm is rarely homogeneous and varies from cell to cell. In some cells, the cytoplasm is finely granular whilst in others, it forms a fine network enclosing numerous small

vacuoles. In some cells, there are discrete, single supranuclear vacuoles but in others, the vacuoles have increased in size to such an extent that the cytoplasm forms only a narrow rim. The surfaces of cells tend to bulge slightly and, in a few, small globular cytoplasmic extrusions are present. Despite the apparent proliferation of the epithelial cells, the mitotic rate is very low.

The crypt zone is very broad, particularly in the longitudinal ridges of the endometrium and merges gradually into the tubular zone (Fig. 51). The crypts are greatly hypertrophied and only a few strands of connective tissue separate them. The cells are very tall and have indistinct borders (Fig. 52). The cytoplasm is completely shattered by cystic irregular vacuolation. The nuclei are small relative to the size of the cells and are distinctly basal in position. In some crypts, the hypertrophy of the cells has excluded the lumen and in these, the nuclei are compressed against the basement membrane. Such nuclei are elongated parallel to the basement membrane and are hyperchromatic. Occasional cells show microcystic vacuolation and the others are surrounded by a clear halo. The mitotic rate is much higher than in the superficial epithelium. Secretion is present in some lumina and in these, the lining epithelium is lower and less vacuolated. The degree of hypertrophy of the crypt zone and the secretory activity are slightly greater in those animals which received oestrogen treatment.

The crypt zone gradually merges into the tubular zone. The cells

become lower and the cell outlines become more distinct. The cytoplasm is less heavily vacuolated and the nuclei are less obviously basal in position. The nuclei are smaller, more crowded and more uniformly orientated at right angles to the basement membrane than those of the crypt zone. Pseudo-stratification is prominent in many of the tubules. The tubules are more hypertrophied and tortuous in their course than those observed in experiment 7. The lumina vary in diameter but only a few are dilated and these are mostly in the oestrogen pre-treated animals. Only a few of the tubules contain secretion.

The R_2 cells zone is the section of the endometrium in which differences due to oestrogen administration are most apparent. In all cases, there is a definite increase in the number of basal glands but the structure of the individual acini varies according to the treatment.

In the non-oestrinised animals, the glands are fairly uniform in diameter and are lined by tall columnar cells closely packed round a lumen the diameter of which approximates to the height of the cells (Fig. 33). The cytoplasm is palely acidophilic and finely vacuolated. Occasional cells contain exhausted vacuoles. The nuclei are smaller, denser and more intensely basophilic than those of the more superficial layers. They are orientated with their long axes at right angles to the basement membrane and so closely packed with a tendency

to pseudo-stratification. Only a few glands contain secretion in their lumina. The mitotic rate is higher than that of the superficial layers or of that of the glandular epithelium in any previous experiment.

In the oestrogen-sensitized animals, there is some variation in the size, shape and structure of the acini. All the glands are larger than their counterparts in the non-sensitized animals. The average glands have wider lumina and a few are moderately dilated. The lining epithelium is taller and distinctly pseudo-stratified with nuclei scattered at any level in the basal halves of the cells. The nuclei vary from round to an elongated oval shape and are more intensely staining than those of the more superficial layers. The cytoplasm is more heavily vacuolated than that of the non-sensitized animals and subnuclear vacuoles are not infrequent. In some of the dilated cystic acini, the cells are much lower and of a cuboidal type. A relatively larger number of acini contain secretion but the proportion is still small.

Glycogen is not detectable in the epithelium in any layer of the endometrium. Granules with the staining characteristics of acid mucopolysaccharide are abundant in the epithelium of the basal glands but are less numerous elsewhere. The secretion present in the lumina has the same staining affinities.

There is no stromal oedema or haemorrhage in the oestrogen treated animals but there are numbers of haemosiderin-containing macrophages which are absent from those which received progesterone alone. The

endometrial stroma is compressed by the hypertrophy of the glandular structures. There is no evidence of abnormal cellular reaction in the stroma.

There are no startling changes in the structure of the myometrium.

Treatment 2.

Four ovariectomised bitches received daily intramuscular injections of 10 mg. of progesterone in oil for 40 days and were then ovariectomised. Queen B28 and B29 each received 5 mg. of stilboestrol dipropionate daily by intramuscular injection for 10 days prior to the start of progesterone treatment. Queen B26 and B27 did not receive any pre-treatment with oestrogens. Queen B28 and B29 both showed signs characteristic of oestrogenic stimulation but these disappeared rapidly under the influence of progesterone. No secondary sex changes resultant on progesterone therapy were observed and the animals remained healthy throughout the experiment.

Haematology.

There was no significant change in the peripheral blood during the period of progesterone administration. Oestrogen effects rapidly disappeared after the termination of the stilboestrol injections.

Biochemistry.

There were no significant alterations in the serum concentrations of bilirubin, calcium, inorganic phosphate and alkaline phosphatase.

the plasma urea levels remained within normal limits throughout the experiment.

Vaginal Cytology.

In cases 128 and 129, the cornification due to oestrogen activity reached its peak in the first few days of progesterone administration and cornified cells rapidly disappeared in the following week. The epithelial cells in all four animals gradually changed in type until the majority were of the small basophil variety. Neutrophils were not observed in significant numbers except in case 129, the smears of which contained a moderate number during the last week of the experiment.

Uterine Anatomy.

The uteri had increased in diameter by approximately 1 cm. during the period of treatment. All contained a small amount of mucoid fluid which failed to yield organisms on cultural examination.

Histology.

The differences in histological appearance between the uteri of those animals which received subcutaneous stimulation and those which did not, are sufficiently great to warrant separate descriptions.

Cases 126 and 127.

The character of the superficial epithelium has changed considerably even that observed in the previous experiments. It is composed of a single layer of tall, stout cells in which the nuclei are

situated in the superficial halves of the cells. The nuclei are irregularly round or oval with wrinkled edges and show no definite orientation. They are deeply staining and some appear to be pyknotic. The subnuclear cytoplasm is stippled with small vacuoles and the cell membranes are distinct. The supranuclear cytoplasm is more intensely eosinophilic and homogeneous so that it is difficult to detect cell membranes. The surface of the epithelium is irregular and secretion is closely adherent to it at various points. No mitoses are present in any of the sections examined.

There is a considerable increase in the number of lumina in the crypt zone and, in parts, there are polypoid projections into the uterine lumen (Fig. 9A). Some of these polypi appear to be merely exaggerations of the normal longitudinal ridges of the endometrium but others are distinctly pedunculated and contain numerous closely packed glandular lumina. The crypts are small with narrow lumina and convoluted walls. The lumina all contain a small amount of secretion. The lining epithelium of the crypts is lower and less staining than that of the surface. The nuclei are situated near the basement membrane and are oval with their long axes at right angles to the surface. The supranuclear cytoplasm is largely homogeneous but vacuoles are present in a few cells. Subnuclear vacuoles are more frequent and occasional nuclei are surrounded by complete halos of vacuolation. Mitotic figures are rare in the crypt epithelium.

There is no obvious increase in the number of tubules most of which are slightly tortuous. The lumina are narrow and all contain a small amount of secretion. The lining epithelium is of the same height as that of the crypt zone but the cells are more closely packed. The nuclei are oval and are close to the luminal surface. The cytoplasm is finely granular and cell boundaries are not distinct. Mitoses are rare.

The number of basal glands in association with each tubule is comparable to that observed in experiment 8. The acini are small with narrow lumina most of which contain secretion. The lining epithelium is composed of low cuboidal cells with oval nuclei situated near and parallel to the luminal surface. The cytoplasm varies in different acini but is generally finely granular. The ductule runs in low.

The endometrial stroma is comparatively dense and cellular. A few neutrophils are present in the superficial stroma of both cases but the numbers are insignificant.

Cases 320 and 322.

The most striking difference is in the structure of the crypt and tubular zones. These have become exaggerated and occupy almost three-quarters of the width of the endometrium. There is a marked increase in the number of glandular lumina present in the combined zones. The lumina are uniformly dilated and are round, oval or even stellate in cross-section. The stroma supporting this network of dilated glands

is reduced to a few coarse collagen fibres compressed between the epithelium lining adjacent lumina. The surface of the endometrium has a scalloped appearance due to the numerous dilated crypt openings (Fig. 59). Highly glandular polyps with slender pedicles project from the crypt zone into the uterine lumen. Some of these polyps are of sufficient size to occlude the lumen and cause compression of the surrounding endometrium.

The epithelium lining the crypts and tubules has become indistinguishable from that of the surface. This epithelium is composed of a single layer of very tall columnar cells. The nuclei are oval with their long axes at right angles to the surface. They are arranged in a regular line in the superficial halves of the cells. The cytoplasm is very pale and cell borders are distinct. Mitotic figures are rare.

There is no obvious increase in the number of basal glands. The acini are mainly small with narrow lumina containing a small amount of secretion. Some have become dilated but, in general, the degree of cystic change is less marked than in the previous specimens. The lining epithelium is composed of low columnar cells with oval nuclei at right angles to the basement membrane.

The endometrial stroma in the basalis zone is unremarkably dense and cellular. There is no evidence of hyperaemia. An occasional neutrophil is present in the superficial epithelium but there is no

evidence of inflammatory reaction.

Experiment 10.

Two ovariectomized bitches, cases D50 and D51, received daily intramuscular injections of 10 mg. of progesterone for 60 days and were then hysterectomized. Both animals received 5 mg. of stilboestrol dipropionate daily by intramuscular injection for 10 days prior to the start of progesterone treatment. Both exhibited the characteristic signs of oestrogen stimulation but these rapidly disappeared under the influence of progesterone. No secondary sex changes were apparent during the period of progesterone therapy and both animals remained healthy throughout the experiment. No evidence of ulcerative pregnancy was observed in the terminal stages of the experiment.

Haematology.

There were no significant changes in the peripheral blood during the period of progesterone administration.

Biochemistry.

There were no significant alterations in the serum concentrations of bilirubin, calcium, inorganic phosphate and alkaline phosphatase. In case D51, the plasma urea level was above normal limits in the later stages but this could be ascribed to a concurrent nephritis.

Vaginal Cytology.

The cornified cells produced by oestrogen stimulation disappeared

within the first 10 days of progesterone administration. Thereafter the smears were similar to those of normal metestrus. Cervical smears taken between the 40th and 50th days of progesterone treatment contained moderate numbers of neutrophils.

Uterine Anatomy.

The uterine horns had increased by almost 1 cm. in diameter during the period of treatment (Fig. 36). Both uteri contained a small amount of clear, mucoid fluid which failed to yield organisms on culture.

Histology.

The basic changes in endometrial structure are very similar in both cases. The normal subdivision of the endometrium in a number of zones in test and gland structure is the same in all levels (Fig. 37).

The superficial epithelium is composed of a single layer of tall, stout columnar cells in which the nuclei are arranged in an irregular line in the superficial halves of the cells (Fig. 38). The nuclei are oval and have triangular borders. They are hyperchromatic and, in some, the aggregation of chromatin is suggestive of pyknosis. The cytoplasm is almost completely vacuolated both above and below the nucleus. In some PAS, there is a narrow homogeneous band of deeply eosinophilic cytoplasm along the surface of the cells. Frozen sections stained with Sudan IV reveal considerable accumulation of lipids in the supranuclear cytoplasm. This type of epithelium bears a

striking resemblance to that seen in the normal bitch in the period 70 - 90 days after coitus.

These are relatively few oviput openings and they are generally small. The character of the epithelium changes abruptly at the edges of the crypts from the tall superficial cells to a low cuboidal type. Small papillomata extend into the uterine lumen but only a few contain glandular elements.

The entire thickness of the endometrium from the superficial epithelium to the inner myometrium is filled with dilated, cystic glands. These cysts vary considerably in size and shape but show the same basic structure at all levels of the endometrium. At some points, it is possible to trace a single glandular tubule or a series of cysts extending from the surface to the myometrium but, in general, the distribution is less regular. Some of the cysts contain small papillomata but these are probably due to the plane of section of coiled tubules. The lining epithelium is composed of a single layer of low cuboidal cells with oval nuclei parallel to the basement membrane (Fig. 59). In some of the larger cysts, the epithelium is of a pavement type with elongated hyperchromatic nuclei. In the majority of the epithelial cells, the cytoplasm is scanty and homogeneous. A few cells contain supranuclear droplets of lipid. Only a few of the cysts contain fluid which is weakly acid mucopolysaccharide in its staining affinity. In a small number of the more superficial cysts, there are occasional neutrophils

in the secretion.

The endometrial stroma forms only a small fraction of the cross-sectional area and is compressed between the cystic glands. The connective tissue is dense and relatively cellular. There are no inflammatory cells in the endometrial stroma but a few scattered hemosiderin-containing macrophages are still present. The endometrium is well supplied with blood vessels but most appear constricted with narrow lumina and thick walls. The endothelial cells of the arterioles are very prominent.

In case 191, a few glandular elements are present deep in the inner myometrium. The vessels of the stratum vasculare are thick-walled and tortuous.

Discussion of Experiments 7 - 10.

These experiments indicate that the response of the endometrium of the young adult bitch to progesterone may be modified by preliminary stimulation with oestrogens.

In those animals which were not sensitized with oestrogens, progesterone therapy induced the normal pattern of histological changes in the endometrium. The epithelium of all zones increased in height and exhibited normal secretory activity with distinct cytoplasmic vacuolation. This secretion was discharged into the glandular lumina in the normal way. Slight structural changes were observed in the crypt and basalis zones but there was no evidence of cystic glandular hyperplasia even after 40 days treatment.

Preliminary oestrogen stimulation produced no obvious morphological differences in the uteri of animals receiving progesterone injections for 10 days but its effect was readily observed in those receiving progesterone for 20 days. In the latter, there was considerable variation in the size, shape and structure of the glands of the basalis zone. The degree of lumen dilatation was in excess of that encountered in any normal healthy endometrium. In the animals subjected to oestrogen treatment followed by progesterone injections for 40 days there was only slight increase of the cystic dilatation of the basalis glands but the crypt zone had undergone structural changes. All the glandular lumina of the crypt zone had become moderately

dilated so that the surface of the endometrium had a pseudo-adenomatous appearance. After 60 days of progesterone treatment, cystic dilatation of the basalis glands was greater and it was no longer possible to distinguish the normal subdivision of the endometrium into zones. The histological picture resembled that of mild cases of natural cystic glandular hyperplasia but lacked the intense proliferative changes of the more severe forms.

To assess the significance of these results, it is necessary to refer back to experiment 4, in which two animals received daily injections of 5 mg. of stilboestrol dipropionate for 10 days. This treatment produced histological changes in the endometrium similar to those of early oestrus but did not cause any dilatation of the glands of the basalis zone. It has been shown that if this period of oestrogen stimulation is followed by daily injections of 10 mg. of progesterone for 40 days or more, a mild form of cystic glandular hyperplasia of the endometrium is produced. This progesterone therapy without preliminary stimulation with oestrogens is incapable of producing such changes. It is apparent that oestrogen stimulation is essential to prepare the glands for a cystic but not for a proliferational response to subsequent progesterone injections. The relationship of oestrogens and progesterone in the pathogenesis of cystic glandular hyperplasia of the canine endometrium is analogous to that of certain chemical carcinogens in that oestrogen acts as an initiator and progesterone as a promotor of cystic dilatation.

Inflammatory changes of the endometrium were not observed in any of the foregoing experiments. This seems at variance with the survey of the cystic hyperplasia-pyometra complex which showed that inflammatory changes appeared only in the luteal phase of the cycle. It may be that the dosage level of progesterone used, though sufficient to induce cystic dilatation, was too low to produce conditions conducive to inflammatory reaction in the endometrium. In the normal diestrus, endometrial edema only in animals of more than three years of age therefore it is unlikely that a single artificial cycle can duplicate the changes produced during at least six control cycles.

Consideration of the survey of the cystic hyperplasia-pyometra complex and of the foregoing experiments suggests that the next logical step in the experimental reproduction of the disease is to study the effect of repeated artificial oestrum cycles on the endometrium.

Group C.

The Effect of Repeated Artificial Cycles on the Endometrium of Ovariectomized Bitches.

Experiment 11.

Two ovariectomized bitches, cases D58 and D55, were submitted to two artificial oestrus cycles. Each cycle consisted of daily injections of 5 mg. of stilboestrol dipropionate for 10 days followed by daily injections of 10 mg. of progesterone for 60 days. The last injection of one hormone and the first injection of the other were on consecutive days. Hysterectomy was performed on the 61st day of treatment. Stimulation of the secondary sex organs was observed during both periods of oestrogen treatment. Both animals remained healthy throughout the experiment.

Haematology.

The peripheral blood exhibited a leucocytosis during both periods of oestrogen treatment.

Biochemistry.

There were no significant alterations in the serum concentrations of bilirubin, calcium, inorganic phosphate and alkaline phosphatase.

Vaginal Cytology.

Cornification of the epithelial cells occurred during both periods of oestrogen stimulation.

Normal Anatomy.

The uterine horns had increased by more than 1 cm. in diameter during the period of treatment. Both uteri contained a small amount of clear mucoid fluid which proved to be sterile.

Histology.

The endometrium is considerably swollen and the uterine lumen has lost its characteristic H-shaped cross-section (Fig. 60). The longitudinal ridges of the endometrium are triangular in N32 but in 933 they are replaced by a larger number of irregular outgrowths some of which are polypoidal.

The superficial epithelium is composed of a single layer of tall columnar cells similar to those observed in experiment 9. The nuclei are placed in the superficial halves of the cells. The cytoplasm is pale and heavily vacuolated. Mitotic figures are not present in any of the sections examined.

There is no obvious increase in the number of crypts when compared to those animals which received only one artificial cycle. The zone has increased in depth. The crypt openings vary from being tightly closed to widely dilated. The lining epithelium is similar in height to that of the surface but the nuclei are distinctly basal in position and the cells are more closely packed. Most of the crypt lumina contain secretion with the staining affinities of acid mucopolysaccharide.

The stroma zone is narrowed except in the bases of the longitudinal

ridges. The tubules are distinctly tortuous. The lumina vary considerably in diameter from narrow clefts to widely dilated cysts, though the majority approximate in width to the height of the lining epithelium. Many of the tubules contain secretion. The epithelium is composed of a single layer of tall, slender columnar cells. The nuclei are closely packed near the basement membrane. In the majority of the cells, the supranuclear cytoplasm is heavily vacuolated.

There is a considerable increase in the number of mitoses in the basalis zone. The glands exhibit an intricate pattern of branching and coiling. The canal associated with individual tubules are formed into groups which are separated from one another by broad intervening bands of stroma. The lumina of the glands vary in diameter from group to group but are generally constant within the group (Fig. 61). Many of the canals are moderately dilated and most of them contain mucoid secretion which has the staining affinities of acid muco-polysaccharide. The lining epithelium is lower than that of the more superficial layers of the endometrium. The nuclei are oval and are arranged in a closely packed line near the basement membrane. The chromatin is coarser and the nuclei are more deeply basophilic than those of the superficial epithelium. The cytoplasm is palely eosinophilic and, in most cells, is homogeneous. Occasional cells contain a few acid muco-polysaccharide granules. Mitotic figures are not uncommon in the lining epithelium.

Hyperplasia of the endometrial stroma has paralleled that of the

glands. Though the endometrium as a whole shows no increase in vascularity, the superficial capillary bed is dilated and the stroma of the crypt zone is oedematous. The reticulin network which is normally present in the crypt zone is prominent but is narrower than that observed in experiment 8. A number of fine collagen fibres are intermixed with the argyrophil fibres along the outer edge of this reticulin network. The periglandular and perivascular reticulin appears unchanged. The collagen fibres of the tubular and basalis zones are coarser and the stromal cells are less numerous.

In the inner myometrium, there is an increase in the number of fine collagen fibres around the muscle cells and a corresponding decrease in the argyrophil fibres. There appears to be a distinct increase in the number and thickness of the collagen fibres in the stratum vasculare. The intermuscular reticulin of the outer myometrium is unaffected.

Experiment 12.

Two ovariectomised bitches, cases D34 and D35, were submitted to four artificial ovarian cycles. Each artificial cycle consisted of daily injections of 5 mg. of stilboestrol dipropionate for 10 days followed by daily injections of 10 mg. of progesterone for 20 days. In case D34 treatment was continuous but an interval of 20 days was allowed between each cycle in case D35. Hysterectomy was performed on the day following the last injection of progesterone in both animals. Both animals

reacted to the oestrogen treatment in each cycle with typical changes in the secondary sex organs. Both animals remained healthy throughout the experiment but D2, showed a 7% loss in weight during the last cycle.

Haematology.

Both animals exhibited a leucocytosis whilst under the influence of progesterone. During the third cycle, D2, exhibited a slight decrease in erythrocyte count and in haemoglobin concentration. The anaemia was further increased during the oestrogen phase of the fourth cycle and normal erythrocyte and haemoglobin levels were not reached until some six weeks after hysterectomy.

Biochemistry.

There were no significant alterations in the serum concentrations of bilirubin, calcium, inorganic phosphate and alkaline phosphatase in either animal during the period of experiment.

Vaginal Cytology.

Cornification of the epithelial cells occurred during all periods of oestrogen.

Genital Anatomy.

In both cases, the uterine horns had increased by over 1 cm. in diameter during the period of treatment. Increase in length was evidenced by distinct tortuosity of the horns which were firm and tense. The lumina were small and contained a trace of clear mucoid fluid which proved to be sterile.

Histology.

The uterine lumen is considerably decreased in size by the swelling of the endometrium. The periphery is contracted and the lumen has lost its characteristic U-shaped outline to become irregularly rectangular or slightly V-shaped. Only small amounts of secretion are present, closely adherent to the superficial epithelium.

The superficial epithelium no longer presents a regular arrangement as in the previous experiment but varies in type with the height of the irregular endometrial ridges. On the peaks of the ridges where crypt openings are few, the cells are low with oval nuclei orientated with their long axes varying from parallel to almost vertical to the basement membrane. The nuclei are relatively small, dense and hyperchromatic. The cytoplasm is scanty and homogeneous. In the troughs formed by the ridges, the epithelium is composed by taller, columnar cells. The nuclei, though similar in structure are more numerous and arranged in a regular row near and vertical to the basement membrane. The cytoplasm is more abundant and bulges to give the cells convex surfaces.

Crypts are most numerous in the angles formed between the endometrial ridges. The mouths of the crypts are wide and shallow. The lining epithelium is much taller with abundant finely vacuolated cytoplasm. The nuclei are larger and more vesicular in appearance with prominent nucleoli. Some cells contain large, single supranuclear vacuoles

and mild muco-polysaccharide is abundant in most.

Though it is still possible to recognize the division between the tubular and basilla zones in most areas, the changes make it more convenient to describe the glandular cysts as a whole, (Fig. 62). All glandular structures are dilated to a much greater extent than those in experiment 11. Some mildly dilated tubules can be traced through a tortuous course to groups of acini in the basilla zone which are dilated to a similar degree. Many tubules dilate gradually towards the basilla zone to end in large irregularly triangular cysts. Some tubules are dilated to form large circular cysts in the middle of the cortex and are usually associated with equally large cysts in the basilla zone. The irregularity of shape of the cysts in the base undoubtedly suggests that many are produced by dilatation and confluence of all the ramifications of individual gland groups, (Fig. 63). The epithelium lining the cystic tubules and basilla glands varies in height inversely with the degree of dilatation. The majority of the cysts are lined by a columnar epithelium of medium height. The nuclei are oval, vesicular and situated near the basement membrane with their long axes at right angles to it. The cytoplasm is palely eosinophilic and vacuoles are not numerous. Correlation with the staining affinities of acid muco-polysaccharide are present in small amounts in the supranuclear cytoplasm of most cells. In the larger cysts, the epithelium has become cuboidal and to cuboidal in type. Only a few cysts contain secretion.

There is a general intensification of collagen deposition with the formation of coarser and denser fibers throughout all strata of the endometrium. In the normal uterus, the collagen fibers are very loosely arranged but in these cases, they are diagnosed as distinctly radial fashion even in the basalis zone where the fibers adjacent to the myometrium normally run a circular course. Though there is no obvious increase in vascularity, the vessels in the endometrium have become much more prominent because of their thickened walls. The perivascular reticulin fibers have been replaced by fine collagen fibers.

The majority of the intramuscular reticulin fibers of the inner myometrium have been replaced by fine collagen fibers. At intervals, the deposition of collagen has been more intense and the muscle is split up into a series of irregular bundles. The perivascular connective tissue of the stratum vasculare is more dense and compact with thickened collagen fibers. In the outer muscle layer, there has been only slight transformation of the reticulin and collagen fibers are relatively infrequent. The perivascular connective tissue shows a considerable increase in the number and thickness of the collagen fibers.

Experiment 42.

Three ovariectomized bitches, nurse D56, D57 and D58, were submitted to six artificial ovarian cycles. Each cycle consisted of daily

injections of 5 mg. of stilboestrol dipropionate for 10 days followed by daily injections of 40 mg. of progesterone for 20 days. In cases 196 and 198, treatment was continuous but an interval of 20 days was allowed to elapse between each cycle in case 197. Hysterectomy was performed on the day following the last injection of progesterone in each animal. All reacted to each course of stilboestrol injections with typical changes in the secondary sex organs. Case 197 remained healthy throughout the experiment. Case 196 showed a 10% loss of weight during the latter half of the experiment. It was inferred that case 198 should undergo eight cycles but she succumbed to an attack of distemper on the 27th day of the ninth cycle.

Haematology.

All three animals exhibited a leucocytosis whilst under stilboestrol treatment. Cases 196 and 198 developed an aplastic anaemia which progressed slowly from the fourth cycle onwards. Both these animals exhibited prolonged bleeding times and high erythrocyte sedimentation rates.

Biochemistry.

There was no significant alterations in the serum concentrations of bilirubin, calcium, inorganic phosphate and alkaline phosphatase in any of the animals. Blood glucose levels were estimated in case 196 and values remained within normal limits throughout the period of experiment.

Vaginal Cytology.

Epithelial cornification was observed during every period of oestrogen stimulation and disappeared rapidly under the influence of progesterone.

Uterine Anatomy.

The uterine horns had increased in each animal during the course of the experiment. The increase in diameter appeared to be due entirely to hypertrophy of the uterine wall because the lumina were reduced to minute proportions. Mucoid fluid was present in small amount in the lumen of case 838 and proved sterile on cultural examination.

Histology.

The atrophic lumina are markedly decreased in size by the swelling of the endometrium and have become irregularly rectangular in shape. Traces of secretion are present in the lumen of case 838 but not in the others.

The superficial epithelium is composed of a single layer of tall, slender columnar cells. The nuclei, which are large and oval are irregularly arranged in the basal halves of the cells. The supranuclear cytoplasm contains a few small scattered vacuoles. Acid mucopolysaccharide granules are present in small numbers in case 838 but are rare in the other cases. Small globules of lipid are present in most of the cells in all cases.

Crypts are most numerous in the troughs formed between the

endometrial ridges which are much less prominent than those in experiment 12. The crypts are V-shaped with wide dilated openings. The lining epithelium is taller than that of the surface with pale cytoplasmic cytoplasm. The nuclei are larger and more vesicular with prominent nucleoli. The nuclei have migrated from the basement membrane and are arranged in a line in the middle third of the cells. Lipids and acid mucopolysaccharide granules are present in a few scattered cells.

The arrangement of the tubular and basilla cells is very similar to that observed in experiment 12 (Fig. 64). The cystic change is more general throughout the entire depth of the endometrium though most of the larger cysts are found in position. The dilation of the cysts into groups related to individual tubules is more distinct because of the growth of the surrounding stroma (Fig. 65). The cysts are mainly orientated with their long axes at right angles to the myometrium probably because of the restricting action of the stroma (Fig. 66). The lining epithelium is generally lower than that observed in experiment 12. Only occasional cysts contain secretion and epithelium is noncecretory.

The connective tissue transformation of the endometrium has progressed further. The entire cytoplasm content of the stroma has become collagenized and only the basement membrane remain acyrophil. The radial distribution of the collagen fibres is more distinct and the

width of these trabeculae has increased.

The myometrium as a whole is thicker than in previous experiments and the inner layer is less clearly demarcated from the adjacent endometrium. This is due to the increase collagen fibres extending into the muscle from the endometrium and obscuring the boundary. The number of collagen fibres around the muscle cells of the inner myometrium is increased. The outer layer is less thickened but collagen fibres are less numerous than reticulin fibres.

Experiment 14.

One ovariectomised bitch, case 333, was submitted to eight artificial ovarian cycles. Each cycle consisted of daily injections of 5 mg. of stilboestrol dipropionate for 10 days followed by daily injections of 10 mg. of progesterone for 20 days. Treatment was continuous and hysterectomy was performed on the 20th day of the experiment. The animal showed a weight loss of 2½ over the period. The coat became dry and dull during the fifth cycle and alopecia became apparent. At first, hair loss was more prominent in the perineal and abdominal skin but eventually the animal was completely bald except on the head and tail (Fig. 67). During the last three cycles, vulval swelling and turgidity was very marked. The animal remained active throughout the experiment but underwent a long convalescence

after hysterectomy.

Hemotology.

A neutrophilia occurred during every period of stilboestrol but the sodium count recorded during each cycle gradually decreased during the later stages of the experiment. During the fourth, fifth and sixth cycles, there was a gradual fall in the erythrocyte count which became constant at just over two million per c. mm. for the remainder of the experiment.

Biochemistry.

There were no significant alterations in the serum concentrations of bilirubin, calcium, inorganic phosphate and alkaline phosphatase. Blood glucose levels were estimated during the last three cycles and remained within normal limits.

Vaginal Cytology.

Epithelial cornification was observed during every period of oestrogen stimulation and rapidly disappeared under the influence of progesterone.

Genital Organs.

The uterine horns had increased in diameter by almost 2 cms. They were externely firm and tense. The uterine lumen would not permit entrance of a 4 mm. probe. The increased thickness of the wall appeared to be largely due to myometrial hypertrophy.

Histology.

The superficial epithelium is composed of a single layer of tall, slender columnar cells. The nuclei are situated in the superficial halves of the cells and give an impression of pseudostratification (Fig. 68). The nuclei are elongated and uniform in appearance. All are deeply basophilic and many are undergoing mitosis. The cytoplasm is pale and contains numerous small vacuoles. Cytoplasmic lipid is abundant.

The crypt zone is narrow but contains a moderate number of gland openings. Most of the crypts are dilated and are lined by an epithelium which is larger than that of the surface. The large vesicular nuclei are mostly basal in position. The cytoplasm contains numerous small vacuoles but only small amounts of lipid are detectable.

It is impossible to differentiate the tubular and basalis zones from one another. The arrangement of the cystic glandular structures is less regular than that observed in specimens 11, 12 and 13 and only a few tubules can be traced from the surface to the myometrium. All the glandular structures are cystic (Fig. 69). The lining epithelium of the cysts is cuboidal or pavement in type with ovoid nuclei orientated parallel to the basement membrane. Mitoses are not present in any of the sections examined. The cytoplasm is homogeneous and no lipid or acid muco-polysaccharide are present. Many of the cyst lumina contain small amounts of secretion which has the staining affinities of acid

muco-polysaccharide.

The collagen deposition which characterised the uterus in the preceding experiment is further increased (Fig. 70). The entire endometrial stroma is composed of relatively acellular coarse connective tissue. The collagen fibres are greatly thickened and have a hyaline-like appearance. The increase has occurred not only in the broad radial band but also around individual cysts which are now scattered. The stromal cells have shrunken, elongated nuclei.

The inner circular layer of the myometrium is grossly thickened. The muscle fibres are interlaced with thick collagen strands which pursue an irregularly circular course. The muscle nuclei are elongated and some are hyperchromatic. The vessels traversing the muscle coat are surrounded by thick layers of collagen fibres. The connective tissue of the stratum vasculare is greatly increased and much of the collagen has become hyalinised. The outer muscle layer is almost as thick as the inner layer and collagen fibres have almost entirely replaced the reticulin network.

Discussion of Experiments 11 - 14.

The results of these experiments can only be assessed in conjunction with experiment 9 which represents the first stage in the series. In the latter, rats B22 and B23 each received 5 mg. of stilboestrol dipropionate daily for 10 days followed by 10 mg. of progesterone daily for 20 days. This treatment produced changes similar to those of the corresponding date of metestrum but with the addition of slight dilatation of some of the basalia glands.

In experiment 11, the animals were submitted to a further cycle of this same type. This produced an increase in the width of the crypt and basalia zones due to hyperplasia of the glands. The arrangement of the basalia glands became more complex and many were moderately dilated.

A complete change occurred in the structural pattern of the endometrium following four artificial cycles. The tubular and basalia zones became confluent and occupied the greater part of the endometrium. All the glandular structures became dilated and presented a picture comparable to that of naturally-occurring cystic glandular hyperplasia. The changes differ from those in experiments 9 and 10 in that the cysts of various sizes are prominent at all levels of the endometrium.

The cystic dilatation of the endometrial glands was maintained throughout six and eight artificial cycles but the pattern was modified

by concomitant changes in the stroma.

After the second artificial cycle there was an obvious increase in the rate of collagen deposition and progressive replacement of the subepithelial and perivascular reticulin networks ensued. In these animals submitted to six or eight cycles, the connective tissue had become heavily collagenised and almost hyaline in appearance. Thick radial strands of collagen fibres compressed the cysts into groups. Gradual decrease in size of the cysts occurred after four cycles.

Similar endometrial fibrosis has been reported in the guinea pig by Lipschutz (1950) and in the rat by Burack, Wolfe and Wright (1942) following prolonged administration of oestrogens. In these animals the final result was atrophy of all glandular structures and complete replacement of the endometrium by coarse fibrous tissue. It seems probable that similar results would be obtained in the bitch with continuation of the type of treatment used in the foregoing experiments.

Inflammatory changes were not observed in any of the uteri submitted to artificial cycles of the type described.

These experiments have proved that cystic glandular hyperplasia can be reproduced by a series of artificial ovarian cycles, each consisting of 5 mg. of stilboestrol dipropionate daily for 10 days, followed by 10 mg. of progesterone for 20 days. As the number of cycles is increased, alterations in the rate of collagen deposition produce a slowly progressive fibrosis of the endometrium. Fibrosis is not a

feature of the cystic hyperplasia-pyometra complex and occurs only in a few chronic cases. This finding plus the absence of inflammatory reaction suggests that the type of artificial cycle used in these experiments is unlikely to produce any of the other forms of the cystic hyperplasia-pyometra complex. It has been shown that the relative proportions of oestrogen and progesterone are of paramount importance in the development of the endometrial and mammary glands in other animals. It seems possible that the oestrogen:progesterone ratio may be a significant factor in the inter-relationship of cystic glandular hyperplasia and endometritis.

When these experiments were completed, two bitches had undergone five cycles of 10 daily injections of 5 mg. of stilboestrol dipropionate followed by 20 daily injections of 10 mg. of progesterone. To test the effect of a higher progesterone:oestrogen ratio, the daily progesterone dose was raised to 50 mg. in the ninth cycle. The results of this change are recorded in experiment 13.

Experiment 13.

Two ovariectomised bitches, cases 140 and 144, were submitted to five artificial ovarian cycles. Each cycle consisted of 10 daily injections of 5 mg. of stilboestrol dipropionate followed by 20 daily injections of 10 mg. of progesterone. The ninth cycle commenced with the normal stilboestrol treatment but, in the subsequent progesterone

plasma, the daily dose was raised from 10 mg. to 50 mg. During each cycle, both animals exhibited the characteristic signs of oestrogen stimulation which rapidly disappeared under the influence of progesterone. Both animals remained healthy throughout the experiment.

Haematology.

Both animals responded to oestrogen treatment with neutrophilia which rapidly disappeared under the influence of progesterone. During the sixth cycle, the leucocyte counts fell to normal levels before the eighth day of progesterone treatment but began to rise again on the 13th day. On the 20th day, both animals had total leucocyte counts of approximately 18,000 cells per c. mm. The leucocytosis was entirely due to an increase in the neutrophil fraction.

Biochemistry.

There were no significant alterations in the serum concentrations of bilirubin, cholate, inorganic phosphate and alkaline phosphatase. In one R/O, the plasma urea level was above normal in the early stages of the experiment but this was due to a concurrent leptospiral nephritis.

Vaginal Cytology.

During the first five cycles, the vaginal cytology showed regular waves of cornification similar to that described in previous experiments. During the sixth cycle, cornified epithelial cells had disappeared by the 10th day of progesterone treatment. On the 12th day, moderate numbers of neutrophils appeared in the smears and persisted until the end of the

experiment.

Genital Anatomy.

The uteri in both animals were swollen and conjested with horns measuring between 2 and 2.5 cms. in diameter (Fig. 71). The lumina contained small amounts of thick yellow mucopus and the carunculae were slightly constricted. The uterine walls were thickened but were less tense and firm than those in experiment 14.

Swabs of the uterine fluid yielded B. coli in case 140 but were sterile in case 141.

Histology.

The uterine morphology differs markedly from that observed in experiments 13 and 14. Despite the obvious swelling of the endometrium, the uterine lumen is enlarged and contains a moderate volume of secretion in which are numerous neutrophils (Fig. 72).

Radical changes have occurred in the structure of the caput zone which is greatly increased in width. The number of glandular lumina and crypt openings have increased and all are dilated so that the zone has a lacunar appearance (Fig. 72). The surface of the endometrium has an irregularly scalloped outline produced by numerous papillary and polypoid outgrowths of the superficial endometrium. The most common outgrowths are those produced by the indentation of the crypts. These are long and slender with only a thin, vascular connective tissue core. Less frequently, there are large polypoid outgrowths with thin

pedicles. These are composed of innumerable cystic glands separated by a relatively thin stroma and have a pseudo-adenomatous appearance.

The superficial epithelium is indistinguishable from that of the crypt zone and is of the florid, highly secretory type with a marked tendency to undergo stratification and focal proliferation. The epithelial proliferation appears as small fan-shaped tufts of cells or as broad sheets of up to six cells thick. The latter form is more frequently observed on the surface. The lumina of many of the cysts have an irregularly stellate outline because of the irregular epithelial proliferation.

The entire crypt zone is diffusely infiltrated with neutrophils and occasional plasma cells. The infiltration is most intense in the superficial stratum and in the surface epithelium. Many of the cysts contain a few inflammatory cells mixed with secretion.

The basal half of the endometrium contains relatively fewer cysts than the crypt zone and they are less regular in size and shape. They are separated off into groups which presumably consist of the cysts arising in individual tubules. Within each group there is considerable variation in size and shape though the basic structure is the same in each case. The lining epithelium is composed of low cuboidal or normal type cells which show no evidence of secretory activity though the cells all contain acid mucopolysaccharide material (Fig. 7). There is little evidence of inflammatory change in the basal half of the

endometrium except a few inflammatory cells in occasional cells. The basic structure of the stroma is similar to that described in experiment 13 with heavy coarse collagen fibres arranged in a radial fashion. The vessels of the endometrium are all dilated and there is evidence of slight oedema. A few of the vessels have perivascular cuffs of plasma cells.

The myometrium exhibits a degree of fibrosis similar to that observed in experiment 13 but the arrangement of the fibres is more definitely circular. The vessels of the mural vasculature are widely dilated.

Discussion of Experiment 16.

The animals in experiments 15 and 16 were subjected to five artificial ovarian cycles each consisting of 10 daily doses of 5 mg. of stilboestrol dipropionate followed by 20 daily doses of 10 mg. of progesterone. There is no reason to suppose that the endometrial reaction differed in the two groups of animals at this stage. In experiment 15, the sixth cycle ran of the same type thereon in experiment 16 the progesterone level was increased to 50 mg. per day. At the end of the sixth cycle, the endometrium of both groups showed cystic glandular hyperplasia but of a very different type. With the lower dosage, the cysts remained comparatively small and their

distribution was modified by fibrosis of the endometrial stroma. In experiment 15, the endometrium responded to the higher dosage with an increase in the complexity of the cystic glandular hyperplasia superimposed on which was acute inflammatory reaction. This was comparable to the milder form of the acute endometritis described in group IX of the survey.

In experiment 15, it has been shown that inflammatory changes will occur in artificially produced cystic glandular hyperplasia of the endometrium when the level of progesterone is raised. If the inflammatory reaction is merely the result of the high dosage rate of progesterone, it should be possible to reproduce an endometritis in a single cycle at this dosage level. However, from the survey of the natural disease, it seems probable that cystic glandular hyperplasia is an essential precursor of this form of endometritis. Since cystic glandular hyperplasia was present in these experimental cases before the increase in progesterone dose, the inflammatory reaction may be due to the change in the testrogen-progesterone ratio. At this stage, it is essential to study the effects of a higher dosage level of progesterone over a number of cycles to elucidate the role of the ovarian hormone in pathogenesis of the disease.

Experiment 46.

Two ovariectomized bitches, cases N#2 and N#3, were submitted to 10 daily injections of 5 mg. of stilbocestrol dipropionate followed by 20 daily injections of progesterone. Case N#2 received 25 mg. of progesterone and N#3 received 50 mg. per injection. Both animals exhibited changes in the secondary sex organs characteristic of oestrogen administration.

Haematology.

The peripheral blood showed the standard reactions to oestrogen administration but these disappeared under the influence of progesterone.

Biochemistry.

There were no significant alterations in the serum concentrations of bilirubin, calcium, inorganic phosphate and alkaline phosphatase during the period of the experiment.

Vaginal Cytology.

Cornification of the vaginal epithelium reached its peak between the third and fourth days of progesterone treatment, regressed over the next five days and thereafter cornified cells were rarely seen. During the course of progesterone treatment, there was a gradual increase in the number of small basophil epithelial cells. Neutrophils were not present in significant numbers during the period of progesterone administration.

Gross Anatomy.

The uteri were slightly enlarged and more rounded than normal. A small amount of mucoid fluid was present in the uterine lumina. Smears of this fluid failed to yield organisms on culture.

Histology.

Despite the difference in dosage into of progesterone, both uteri exhibit the same histological features and show only slight intensification of the secretory changes observed in experiment 8 where the dosage level was only 10 mg. daily.

The superficial epithelium is tall columnar in type with the nuclei closely packed in the middle third of the cells. The nuclei are large, oval and pale staining. The cytoplasm of most cells forms a fine network enclosing numerous small vacuoles. In a few cells, the cytoplasm is completely displaced by single large vacuoles.

The crypt zone is increased in depth and the crypts are large and prominent (Fig. 75). The lining epithelium is composed of very tall columnar cells with relatively small nuclei in the basal position. The cytoplasm is completely disrupted by vacuolation and cell borders are indistinct. Secretion is present in a few of the lumina but in most of the crypts, acid muco-polysaccharide is entirely intra-epithelial.

The crypt zone merges gradually into the tubular zone in which the lining epithelium is much lower. The cell nuclei are smaller and more deeply staining. They are closely packed near the basement membrane.

The diameter of the tubules varies to a certain extent but there is no marked dilatation. Only a few tubules contain secretion.

There is some variation in the size, shape and structure of the cells of the basal zone. Most of the glands have wider lumina than normal and a few are moderately dilated. The lining epithelium is taller than that of the tubules and is pseudo-stratified in many glands. The nuclei are larger and more deeply staining than those of the tubular epithelium. The cytoplasm is only mildly vacuolated and acid muco-polysaccharide granules are not numerous. Only a few acini contain secretion.

The endometrial stroma is comparatively dense and cellular with no evidence of oedema or haemorrhage. There are no inflammatory changes in any part of the endometrium.

Experiment 17.

Two ovariectomised bitches, ranges 17A and 18B, were submitted to two artificial ovarian cycles. Each cycle consisted of daily injections of 5 mg. of stilboestrol dipropionate for 10 days followed by daily injections of 25 mg. of progesterone for 20 days. Hysterectomy was performed on the first day of treatment. Stimulation of the secondary sex organs was observed during both periods of oestrogen administration. Both animals remained healthy throughout the experiment.

Pronestrous.

Leucocytosis was observed during both periods of oestrogen treatment

but disappeared under the influence of progesterone.

Biochemistry.

There were no significant alterations in the serum concentrations of bilirubin, calcium, inorganic phosphate and alkaline phosphatase during the experiment.

Vaginal Cytology.

Cornification of the vaginal epithelial cells occurred during both periods of oestrogen stimulation. Under the influence of progesterone the cell picture rapidly changed to that of early metoestrus.

Morbid Anatomy.

The uteri had increased by almost 1.5 cm. during the period of the treatment. The uterine horns were firm and tense. The lumina contained a small amount of mucoid fluid which proved sterile on culture.

Histology.

The endometrium is swollen and the uterine lumen is reduced to a narrow X-shaped slit (Fig. 76).

The surface epithelium is of a tall columnar type with nuclei situated in the superficial halves of the cells (Fig. 77). The cytoplasm is palely eosinophilic and contains numerous tiny vacuoles both above and below the nucleus. Acid muco-polysaccharide granules are present in small numbers in the supranuclear cytoplasm and there are lipid droplets in the subnuclear cytoplasm. Mitoses are not present in any of the sections examined.

The crypt zone has become wider and there is a distinct increase in the number of glandular lumina present. The crypt openings and the adjacent lumina are uniformly dilated and are lined by an epithelium similar to that of the surface. The cells are more closely packed but exhibit the same secretory activity. The stroma supporting this network of dilated glands is reduced to a few collagen fibers compressed between the epithelium lining adjacent lumina.

The width of the tubular zone differs in the two animals. In case 124, it is considerably narrower than in 125 it appears to have increased in width. The structure of the tubules is the same in both animals. The lining epithelium is composed of a single layer of tall, slender columnar cells with basal nuclei. The cytoplasm is heavily vacuolated but contains no lipid. Acid mucopolysaccharide material is abundant in most cells. The lumina of the tubules vary in diameter from narrow slits to dilated cysts but the majority are moderately dilated. Many of the tubules dilate gradually towards the basilar zone to end in large irregular cysts so that the normal zone boundary is no longer distinct.

The number of acini in the basilar zone is increased and the tubules terminate in an intricate pattern of branching and coiling. Most of the acini are dilated to a greater extent than those observed in experiment 11 where the progesterone dose was 10 mg. per day. The lining epithelium is composed of low columnar or cuboidal cells with

deeply basophilic nuclei. The cytoplasm of most of the cells is homogeneous and acid mucopolysaccharide granules are scanty. Most of the cysts contain secretion.

Changes in the endometrial stroma are less obvious than in experiment 14. The crypt and tubular zones in both cases are oedematous. There is no obvious reduction in the size of the sub-epithelial and perivascular reticulin networks of the endometrium but the collagen fibres of the basilar zone are noticeably coarser.

The reticulin network of the myometrium shows no evidence of collagen deposition. The vessels of the stratum vasculare are prominent and the perivascular connective tissue is slightly oedematous.

Experiment 16.

Three ovariectomised bitches, cases D46, D47 and D48 were submitted to four artificial ovarian cycles. Each cycle consisted of 10 daily injections of 5 mg. of dihydroxyoestrone followed by 20 daily injections of 25 mg. of progesterone. In cases D46 and D47, the treatment was continuous and hysterectomy was performed on the 42nd day of the experiment. In case D48, an interval of 20 days without treatment was allowed between each cycle and hysterectomy was performed on the 101st day.

On the 17th day of the progesterone phase of the third cycle

case D&F refused all food and was obviously ill. Her temperature was 105.5°F but returned to normal by the following morning when she was considerably brighter. On the third day of ostilbestrol treatment in the fourth cycle, there was a moderate volume of purulent discharge from the vulva and the animal returned to normal. On the 10th day of the progesterone phase of the fourth cycle, symptoms reappeared unexpectedly, initially, by vomiting. The animal remained dull until the end of the experiment.

Cases D&7 and D&8 remained healthy until the last 10 days of the fourth cycle when both developed symptoms similar to those of case D&6.

Haematology.

In case D&6, the leucocytosis in response to ostilbestrol administration in the third cycle fell to within normal limits by the 10th day of progesterone treatment. The total white cell count began to rise on the 16th day of progesterone treatment and the leucocytosis was maintained throughout the subsequent ostilbestrol phase of the fourth cycle. The leucocyte count then fell to normal proportions by the eighth day of the progesterone phase of the fourth cycle and began to rise again on the 10th day to reach a maximum of 35,000 cells per c. mm. on the 16th day.

No leucocytosis was observed during the progesterone phases of the first three cycles in cases D&7 and D&8. During the progesterone phase of the fourth cycle the leucocytosis induced by ostilbestrol

treatment had disappeared by the eighth day in both animals. Leucocytosis again became apparent in case N$7 on the 10th day and in case N$8 on the 12th day. Both animals had total leucocyte counts of over 20,000 cells per c. mm. on the last day of the fourth cycle.

Biochemistry.

There were no significant alterations in the serum concentrations of bilirubin, calcium, inorganic phosphate and alkaline phosphatase during the period of experiment. The plasma urea level rose slightly during the initial stages of the second illness in case N$5.

Vaginal Cytology.

The differential vaginal cell counts followed the same pattern as in the previous experiment during the first two cycles in all three animals. In case N$5, abnormal numbers of neutrophils appeared in the smears during the last few days of the third cycle and reached a maximum on the third day of the stilboestrol phase of the fourth cycle. There were no changes in the cellular pictures in the other two animals during the third cycle. In all three animals, during the fourth cycle, moderate numbers of neutrophils appeared in the smears taken on the day prior to the onset of illness and persisted until the end of the experiment.

Morbid Anatomy.

The uterine horns of case N$5 were both approximately 25 cms. long and were of an almost uniform diameter of 3.5 cms. throughout their

length. The uteri in the other two cases were only slightly less enlarged but differed from case M5 in the form of distension. In both cases there were a series of annular constrictions which produced a number of short sausage throughout the length of the horns (Fig. 78). In case M7, the posterior half of the left horn was narrower than the anterior half. The corpus uteri in all three cases was less obviously enlarged and did not exceed 2 cms. in diameter. The cervices were tightly constricted and would not permit passage of a 3 mm. probe. The lumina of the uteri contained a considerable volume of thick, yellow-green muco-pus which, in parts, was firmly adherent to the endometrial surface. The endometrium in each case had a roughened appearance and the cysts were of a dull yellow colour (Fig. 79). In case M6, there was evidence of ulceration and focal haemorrhage.

Cultural examination of the pus yielded B. coli in cases M5 and M8. No organism was isolated from the uterine fluid of case M7.

Histology.

The basic lesion in all three cases is an acute endometritis superimposed on cystic glandular hyperplasia but there is sufficient difference in severity and character of the changes to warrant a separate description of each case.

Case M8.

The glandular elements of all strata of the endometrium are

dilated and cystic. Though the cysts vary greatly in size and structure they are divided into two fairly distinct groups (Fig. 80). The superficial cysts appear to have arisen by proliferation of the crypts and adjacent tubules and are mostly lined by an epithelium similar to that of the surface. These cysts are generally large and very irregular in outline. Some project into the uterine lumen on slender pedicles. The epithelium is of a florid, highly secretory type exhibiting considerable variation in arrangement even within the individual cysts. The epithelial cells are mainly very tall with pale, oval nuclei varying in position from the basement membrane to the surface. The cytoplasm is palely eosinophilic and contains numerous tiny vacuoles. Some cells are completely vacuolated and appear as goblet cells or as intra-epithelial cysts. Many of the cells have small superficial cytoplasmic protrusions which may be tuft-like or attached by slender pedicles. The cells may be arranged in a single layer but often the arrangement is more complex (Fig. 81). Pseudo-stratification is commonly confined to small areas which form tuft-like projections into the lumina. Particularly on the endometrial surface, the epithelium forms syncytium-like sheets in which cell membranes are indistinct and vacuolation of the cytoplasm is prominent. This irregular proliferation of the epithelium gives a finely scalloped border to the cysts and surface of the endometrium.

Production of secretion in the crypts and superficial cysts seems

extensive and stroma of mucin appear to flow from individual cells. The amount of intra-cytoplasmic acid muco-polysaccharide material varies greatly from cell to cell.

The cysts in the basal half of the endometrium are mainly smaller though a few almost span the entire width of the endometrium. Most of these cysts are oval or kidney-shaped with their long axes orientated at right angles to the uterine lumen. The epithelium lining the majority of the cysts is of a non-secretory cuboidal type. A few are lined by low, pavement-type cells and occasionally, by tall secretory columnar cells. Most of the cysts contain secretion which has the staining affinities of acid muco-polysaccharide.

The epithelium of the surface and crypts is heavily infiltrated by neutrophils which appear in many instances to be within the epithelial cells. The neutrophils are normally surrounded by a clear halo. Neutrophils are numerous in the stroma of the crypt zone which is congested and oedematous. Exudation of inflammatory cells into the crypts and cysts is irregular and whilst some contain only mucin, others are filled with neutrophils and cell debris. A few plasma cells and macrophages are present in the stroma around the cysts.

The basal half of the endometrium is very oedematous and congested but is free from any cellular infiltration. The collagen fibres are thickened and coarse but are widely separated by the oedema fluid. The periglandular reticulin network is thin and compressed.

The muscle cells of the myometrium are slender and elongated and the reticulin network is more definitely circular in arrangement. The stratum vasculare is oedematous and the vessels are prominent.

Case P47.

The endometrium has undergone cystic glandular hyperplasia which is similar to that observed in case P40. It differs from the latter only in the extent and severity of the inflammatory reaction.

The inflammatory changes are more diffuse and involve the entire width of the endometrium (Fig. 82). The process is more severe in the crypt zone where numerous small abscesses are present (Fig. 83). The superficial epithelium overlying some of these abscesses is undergoing degenerative changes and occasionally, ulceration has occurred. These ruptured abscesses appear to become confluent with the exudate in the uterine lumen. Small focal hemorrhages occur in close proximity to some of the ulcers. Despite the heavy concentration of neutrophils in the stroma of the crypt zone, many of the cyst lumina do not contain inflammatory cells.

The stroma of the basal half of the endometrium is oedematous and diffusely infiltrated with neutrophils. The majority of the cysts contain cellular exudate and, in many, the lining epithelium has become necrotic with the formation of abscesses (Fig. 84). Some cysts contain no inflammatory cells despite the heavy concentration in the surrounding

areas.

Some of the abscesses have extended to involve the inner layer of the myometrium resulting in necrosis of the muscle fibres (Fig. 85). At some points, lines of neutrophils and macrophages can be seen extending along between groups of muscle fibres. Some extension of the acute inflammatory reaction has occurred along the perivascular connective tissues of the inner myometrium. A few adenomyotic foci are present and all are involved in the general inflammatory reaction. The stratum vasculare is oedematous and the vessels are dilated and congested.

Case H6.

The endometrium has undergone cystic glandular hyperplasia as in the previous case. There is a diffuse infiltration of neutrophils throughout the endometrium accompanied by abscess formation and ulceration (Fig. 86). The inner myometrium is more extensively involved in the acute inflammatory process. In addition, there is a diffuse infiltration of plasma cells which are most numerous in the stroma of the crypt zone. In the basal half of the endometrium and in the myometrium, the plasma cells are present in the form of perivascular cuffs. The distribution of the plasma cells is very similar to the distribution of the neutrophils in case H5. The structure and arrangement of the connective tissue does not differ from that in the other two cases.

Discussion of Experiments 16 - 18.

The animals in these experiments were submitted to a series of artificial ovarian cycles in the progesterone phase of which a daily dose of 25 mg. was given. The results are more readily assessed if they are considered in conjunction with experiments 11 - 14 in which a daily dose of 10 mg. of progesterone was administered.

The changes produced by a single cycle varied little with the difference in the level of progesterone. The second cycle produced a degree of hyperplasia and dilatation of the crypts and basalis glands comparable with that obtained with four cycles at the lower progesterone level.

All the three cases in experiment 18 which were submitted to four cycles responded with marked cystic glandular hyperplasia and acute inflammatory changes. These lesions are similar in every respect to those observed in Group III of the natural disease. Clinical symptoms of genital disease became apparent in the progesterone phase of the third cycle in case N.5 and at the same stage of the fourth cycle in all three animals. Further evidence that the inflammatory reaction is progesterone dependent is afforded by the fact that the withdrawal of progesterone at the end of the third cycle led to discharge of uterine fluid and alleviation of symptoms in case N.5.

These results show that the endometrium must be subjected to a number of cycles at a high progesterone level before endometritis will

occur. The endometritis only becomes apparent during the progesterone phase and symptoms disappear on withdrawal of this hormone. In case M46, it was noted that attacks of illness occurred in the third and fourth cycles whereas the other animals only reacted in the fourth cycle. The endometrial histology of case M46 differed from that of the others in that plasma cells were numerous in the stroma. It seems possible that the plasma cells were part of a repair or reactionary process following the first attack of endometritis. Some additional support to this hypothesis is afforded by the fact that the plasma cells were distributed in a similar manner to the neutrophils in case M48 which suffered only one attack.

Further study of this plasma cell reaction may give some indication of the position of the Group II lesion in the sequence of pathogenetic changes in the cystic hyperplasia-pyometra complex. The next experiment was undertaken to study the effect on the endometrium of withdrawal of progesterone following induction of endometritis.

Experiment 19.

Two ovariectomised bitches, cases M51 and M52, were submitted to four artificial ovarian cycles. Each cycle consisted of 10 daily injections of 5 mg. of stilboestrol dipropionate followed by 20 daily injections of 25 mg. of progesterone. Case M51 became ill on the 11th day and M52 on the 13th day of the progesterone phase of the fourth

cycle. On the last day of the fourth cycle radiographs of the abdomen were taken. The animals were then left without treatment of any kind for 15 days before being hysterectomised. Both animals exhibited a profuse muco-purulent vulval discharge during the fourth and fifth days after the termination of progesterone. The listlessness and inappetence which characterised the illness disappeared with the onset of vulval discharge and the animals remained healthy until the end of the experiment. Radiographs taken just prior to hysterectomy revealed that considerable reabsorption had occurred in the mass of the uteri during the 15 days after the termination of treatment.

Haematology.

In case B91, the leucocytosis in response to stilbocestrol administration in the fourth cycle fell gradually until the eighth day of progesterone treatment. The total leucocyte count then began to rise to reach a maximum of 25,000 cells per c.mm. on the last day of treatment and remained of this order for the next four days. When vulval discharge became apparent, the white cell count dropped dramatically and ran within normal limits for the last five days before hysterectomy.

Case B92 showed a similar leucocyte response to onset of symptoms and a similar return to normal following the onset of vulval discharge.

Biochemistry.

There were no significant alterations in the serum concentrations of bilirubin, calcium, inorganic phosphate and alkaline phosphatase

during the period of experiments. In addition, serum protein estimations were carried out from the beginning of the last cycle until the animals were hysterectomized. In both animals, an increase in the serum protein level occurred during the last three days of the progesterone phase and was maintained until the tenth day after termination of treatment. The albumin/globulin ratios were reduced, particularly during the recovery period after discharge of the uterine contents.

Vaginal Cytology.

The vaginal cell picture followed the normal cyclic pattern until the day prior to the onset of illness when numbers of neutrophils appeared. The neutrophils were not particularly numerous until the second day after the termination of treatment. On that day and for the subsequent three days, the numbers of neutrophils were such as to obscure completely the epithelial picture. Thereafter, the number of neutrophils rapidly decreased and the final smears were of the type observed in the late metoestrus and early anoestrus.

Morbid Anatomy.

The uterine horns, though enlarged were much smaller than those observed in experiment 16. They were flaccid and the longitudinal ridging of the peritoneal surface suggested they had been previously dilated as the radiographs showed. The cervices were open and the uterine lumina contained only a small amount of pale, slightly turbid

mucoid fluid. The surface of the endometrium had greyish, somewhat wrinkled appearance. There was no evidence of ulceration or hemorrhage.

Cultural examination of the pus yielded B. coli in both cases.

Histology.

The changes in both cases are similar and a composite description is given. The endometrium has undergone cystic glandular hyperplasia which is similar to that observed in the preceding experiment (Fig. 88). The cysts are generally smaller but are still divided into two distinct groups. The epithelium of the endometrial surface and superficial cysts is of the flood, highly secretory type with a marked tendency to form pseudo-papillomatous proliferations. The cysts of the basalis zone are lined by a cuboidal epithelium. All the lumina contain a variable amount of secretion which is cell-free except in a few superficial cysts where small numbers of neutrophils remain.

The cellular infiltration of the endometrium is almost entirely composed of plasma cells with a few lymphocytes and macrophages (Fig. 89). A few neutrophils are present within the surface epithelium but are rare in the endometrial stroma. The distribution of the plasma cells is similar to the distribution of neutrophils in the experimental cases of acute endometritis. They are most numerous in the superficial half of the endometrium and gradually decrease towards the inner myometrium. The vessels of the inner myometrium are surrounded by cuffs of plasma cells.

There is no evidence of necrosis or atrophy of any endometrial structure. The degree of collagen deposition is comparable to that observed in experiment 18. The basalis region of the endometrium is congested and oedematous.

Discussion of Experiment 19.

From a comparison of experiments 18 and 19 it seems justifiable to conclude that cases D21 and D22 indicate endometritis during the latter half of the progesterone phase of the fourth cycle. Withdrawal of progesterone treatment resulted in clinical recovery which lends further support to the contention that the endometritis accompanying cystic glandular hyperplasia is progesterone dependant. Examination of uterine fluid following termination of treatment indicates the role of progesterone in the control of the cervix. In the experimental cases of acute endometritis which were under the influence of progesterone when examined, the cervix was firmly closed.

Though it is certain that the animals had had an acute endometritis, when the uteri were examined 19 days after suspension of treatment a complete change had occurred in the endometrial histology. The neutrophils had disappeared and were replaced by plasma cells so that the endometrium simulated those in Type II of the natural disease. It would appear that this lesion is part of the natural recovery processes following an acute inflammatory reaction in the endometrium.

All the clinical and histological findings in the natural condition support this contention. The natural lesion is observed at a later date in metoestrus than acute endometritis and the cases commonly have a history of recent genital discharge. There is some circumstantial evidence of lowered progesterone output. The cervix is always relaxed in these cases and the corpora lutea are undergoing retrogressive changes.

From the results of this experiment, it is suggested that the type II lesion of the cystic hyperplasia-pyometra complex in the bitch is not a specific inflammatory reaction but is a repair or reactionary process following spontaneous recovery from an acute endometritis.

Experiment 20.

Two ovariectomised bitches, cases 149 and 150, were submitted to four artificial cycles. Each cycle consisted of 5 mg. of stilboestrol dipropionate daily for 10 days followed by 20 days without treatment. Hysterectomy was performed on the 121st day of the experiment. Stimulation of the secondary sex organs was observed during each period of oestrogen treatment. Both animals remained healthy throughout the experiment.

Haematology.

Leucocytosis was observed during each period of stilboestrol treat-

ment and the total leucocyte counts returned to normal in the intervals.

Vaginal Cytology.

Cornification of the vaginal epithelial cells occurred during the periods of stilbestrol injections and slowly disappeared during the intervals without treatment.

Genital Anatomy.

The uterine horns were thin and rounded but only slightly increased in diameter. There was no change in the uterine lumen.

Histology.

The superficial epithelium is composed of a single layer of low columnar cells. The nuclei are oval, deeply staining and are situated close to the basement membrane. The cytoplasm is homogeneous and does not contain cold muco-polysaccharide or lipid. Mitotic figures are not present in any of the sections examined.

There is no obvious increase in the number of crypts which are shallow and U-shaped. The epithelium is only slightly taller than that of the surface and is non-secretory.

The tubular zone is broad but contains relatively few glands. The lumina are slightly dilated but do not contain secretion. The lining epithelium is slightly lower than that of the surface and is non-secretory.

There is a considerable increase in the number of glands in the basal zone. Many of them are branched. The lumina are moderately

dilated but do not contain secretion. A few are cystic. They are lined by a low cuboidal epithelium which is non-secretory (Fig. 90).

There is no evidence of focal haemorrhage or hyperaemia of the endometrium. There is a marked transformation of the connective tissue of the endometrium. The reticulin networks of the endometrium have become collagenised and only the basement membranes remain argyrophil. The collagen fibres are thick and coarse and are arranged in a radial fashion in the form of trabeculae between groups of glands.

The myometrium is thickened and the reticulin network is almost completely collagenised. The vessels of the stratum vasculare have very thick walls.

Discussion of Experiment 20.

This experiment was performed in order to give a clearer definition of the effects of stilboestrol and of progesterone in the previous experiments. It is evident that oestrogen causes a moderate increase in the number of glands in the basalis zone but produces only slight dilatation. It can be assumed that in the previous experiments the dilatation was produced as a direct effect of progesterone on glands sensitised by oestrogen. It is important to note that inflammatory changes did not occur in the absence of progesterone. The degree of collagen deposition is more advanced in the present experiment than in

those receiving additional progesterone treatment. It can be concluded from this that oestrogens have a fibroneogenic action on the canine uterus similar to that described by Lipschutz (1950) in the guinea-pig.

Experiment 21.

Two bitches, cases 853 and 854, were ovariectomised the left horn of the uterus in each one ligated near its union with the corpus uteri and was then transected. Care was taken to retain the normal vascular channels of the isolated horn. One month later, each animal received 10 daily injections of 5 mg. of stilboestrol dipropionate followed by 20 daily injections of 25 mg. of progesterone. Both animals remained healthy throughout the experiment et the end of which they were hysterectomised.

Haematology.

The leucocyte response to oestrogen administration disappeared rapidly under the influence of progesterone and no further changes occurred in the peripheral blood during the experiment.

Vaginal Cytology.

The epithelial cornification elicited by stilboestrol was replaced by an early metestrual picture during the period of progesterone administration.

Horned Antlers.

In each case, the left horn was about twice the diameter of the right horn (Fig. 94). The former was thin-walled and contained a considerable volume of mucoid fluid. The right horn had a narrow lumen and contained only a small amount of fluid. The contents of both horns proved to be sterile on cultural examination.

Histology.

The morphological features of the right horn of the steers are similar to those described in experiment 16.

The lumen of the left horn is greatly increased in diameter and the endometrium is compressed so that it is merely here than one cell in depth (Fig. 92). The luminal surface of the endometrium is smooth and regular with only occasional indentations which are probably remnants of crypts. The superficial epithelium is composed of tall columnar cells with basal nuclei which are large and oval. The cytoplasm contains only a few small vacuoles and is deeply eosinophilic.

The antral are of similar size to those in the right horn but show no definite arrangement. The endometrium is so thin that many glands are in contact with the superficial epithelium and with the myometrium. They are lined by a very tall columnar epithelium. The nuclei are smaller and more deeply staining than those of the surface. The cytoplasm is heavily vacuolated and palely eosinophilic.

The endometrial stroma is dense and the fibres are arranged in a distinctly circular manner. Blood vessels are numerous but show no evidence of congestion. There are no abnormal cells in the stroma.

The myometrium is dense and compressed. The vessels of the stratum vasculare are prominent but are not congested.

Experiment 22.

When cases 955 and 956 were ovariectomised the left horn of the uterus was isolated by ligation and was transected near its union with the corpus uteri. After an interval of one month, they were submitted to four artificial ovarian cycles, each consisting of 10 daily injections of 5 mg. of stilboestrol dipropionate followed by 20 daily injections of 25 mg. of progesterone. During the last 10 days of the fourth cycle both showed inappetence, dullness and pyrexia. Case 955 was hysterectomised on the day following the last injection of progesterone whereas hysterectomy was delayed for 15 days in case 956. Discharge of uterine fluid occurred three days after the last progesterone injection in case 955 but the animal showed only slight improvement in condition.

Haematology.

The changes in the blood picture followed the normal cyclic pattern until the onset of illness when both animals exhibited a leucocytosis. In case 956, the total leucocyte count fell slowly

after discharge of uterine contents but did not return to normal before hysterectomy.

Vaginal Cytology.

Normal cyclic changes were observed in the vaginal smears until the day prior to the onset of illness when numbers of neutrophils appeared. The neutrophil content of the smears remained high until the end of the experiment in both animals.

Gross Anatomy.

The left horn in both cases was approximately 4 cm. in diameter. In case 255, the right horn measured almost 3 cm. in diameter whilst in 256 it was less than 2 cm. The isolated horns were thin-walled and contained a considerable volume of watery mucopus. The right horns both contained thick yellow mucopus. Cultural examination revealed the presence of B. coli in the fluid of all four horns.

Histology.

Case 255.

The endometrium of the right horn exhibits an acute inflammatory reaction superimposed on cystic glandular hyperplasia similar to that of experiment 40.

In the left horn, the endometrium is greatly narrowed so that there are very few glands. The surface of the endometrium is generally smooth with occasional indentations (Fig. 93). The superficial epithelium is composed of tall columnar cells with nuclei

situated near the middle of the cell. The glandular cytoplasm is heavily vacuolated. Areas of superficial ulceration are present and there is diffuse cellular infiltration of the epithelium and stroma. The cells are mainly neutrophils with a mixture of plasma cells, macrophages and a few lymphocytes. The endometrial stroma is dense and is arranged in a circular fashion. The myometrium is obviously compressed and there is some extension of the inflammatory process along the perivascular connective tissue.

Cow D55.

The histological changes in the right horn which had five days oxe similar to those observed in experiment 19. The endometrium has undergone cystic glandular hyperplasia superimposed on which is a diffuse infiltration of plasma cells.

In the distended left horn, the endometrium is even narrower than in case D53. The superficial epithelium is intact and there is no evidence of ulceration. It is composed of low cuboidal cells which show no secretory activity. Glandular elements are scanty and tend to be scattered in small groups around the endometrium (Fig. 9). The acini are small and are lined by an epithelium similar to that of the surface. The stroma of the endometrium is dense and difficult to distinguish from the inner myometrium because of atrophy of the latter. The endometrium and the adjacent myometrium are diffusely infiltrated by lymphocytes and plasma cells. Occasional macrophages

and mast cells are present but neutrophils are rare except in the uterine exudate.

Many of the muscle fibres of the inner myometrium are atrophic and have lost their staining affinity. The nuclei are slender, elongated and hyperchromatic. The vessels of the inner muscle layer and the stratum vasculare are surrounded by cuffs of lymphocytes and plasma cells.

Discussion of Experiments 21 and 22.

These experiments illustrate the effects on the uterus of complete retention of the fluid products of the endometrium. The initial changes are accumulation of secretion and consequent dilatation of the uterus with compression of the endometrium. After four artificial cycles, inflammatory changes supervened. The inflammatory reaction was similar in nature to that observed in the experimental cases of acute endometritis. However, on withdrawal of progesterone treatment the cellular infiltration of the endometrium altered in composition and the neutrophils were replaced by lymphocytes and plasma cells. The endometrium showed marked resemblance to those grossly distended cases described in Group IV of the natural disease.

It seems possible that the distended uteri described in Group IV are formed by continued retention of uterine fluid after an attack of

acute endometritis at an earlier stage in the cycle. It is known that most of these animals not only had attacks of endometritis at earlier stages in the cycle but also during previous cycles. Retention of uterine fluid can be accounted for by the fibrotic changes in the lamina propria of the cervix. Since the cervix shows inflammatory changes in acute endometritis it is not improbable that fibrosis will occur after several attacks.

Discussion and Conclusions.

A survey of the cystic hyperplasia-pyometra complex revealed that it is a disease of the older nulliparous bitch which may become clinically manifest as an endometritis during the metestanal phase of the ovarian cycle. On a histological basis, it was possible to divide the cases into four broad groups. A gradation of the histological changes could be traced from uncomplicated cystic glandular hyperplasia of the endometrium through acute endometritis to a plasma cell endometritis and thence to a chronic endometritis. It was possible to correlate ovarian changes with those in the endometrium. Uncomplicated cystic glandular hyperplasia was observed at all stages of the cycle. In acute endometritis, the morphological appearance of the corpora lutea indicated that they were always active even at the stage of the cycle when they should normally have regressed. The plasma cell reaction was observed only when the corpora lutea were regressing. In the

majority of the diestrus cases, the corpora lutea had regressed though a few were active long after the normal period of regression.

From this survey, it was concluded that the etiology and pathogenesis of the cystic-hyperplasia-pyometra complex are intimately related to the functional state of the ovarian. This contention was supported by a series of experiments to study the effects of ovarian hormone on the endometrium of the ovariectomized bitch. By various combinations of oestrogens and progesterone it was possible to reproduce the principal form of the cystic hyperplasia-pyometra complex.

Oestrogens in daily doses of 5 mg. - 20 mg. per day for up to 40 days failed to produce cystic glandular hyperplasia. Progesterone in daily doses of 10 mg. produced only metoestral changes but when it was used with preliminary oestrogen stimulation a mild form of cystic glandular hyperplasia of the endometrium was produced. It is concluded from these experiments that oestrogen stimulation is essential to prepare the glands for a cystic but not for a metoestral response to subsequent progesterone injection.

Since the natural disease requires several oestrus cycles in which to develop, it was essential to simulate these by alternate treatment with oestrogen and progesterone. The first cycle used consisted of 10 daily injections of 5 mg. of stilboestrol dipropionate followed by 20 daily injections of 10 mg. of progesterone. After four such cycles, the degree of cystic glandular hyperplasia was comparable to that

occurring in natural cases. However, the histological picture was supplanted by increased deposition of collagen and atypical fibroids which progressed with each subsequent cycle.

In one animal the dosage rate of progesterone was increased from 10 mg. to 50 mg. per day in the sixth cycle. This animal developed an acute endometritis similar to that described in Group III of the survey. This indicates that the progesterone level required to produce inflammatory changes is relatively higher than that required to produce cystic glandular hyperplasia. That cystic glandular hyperplasia is an essential precursor of acute endometritis was shown by the failure of a single cycle of this type to produce inflammatory changes.

Using cycles in which the progesterone dose was 25 mg. per day cystic glandular hyperplasia of the endometrium was produced after two cycles and acute endometritis appeared after four cycles. If progesterone was withheld there was relief of symptoms and spontaneous discharge of the uterine contents. In the endometrium the acute inflammatory reaction regressed and was replaced by a plasma cell infiltrate.

It was possible to reproduce the severe form of chronic endometritis by a combination of hormonal treatment and surgical interference with uterine drainage. If one uterine horn was isolated, an acute endometritis developed in both horns during the fourth artificial cycle. Progesterone was withheld after the onset of the inflammatory

reaction and the reaction began already in the isolated horn.

The experiments indicate the possible pathogenetic sequence of changes in the cystic hyperplasia-pyometra complex. It is evident that cystic glandular hyperplasia of the endometrium develops during a number of non-pregnant cycles under the influence of oestrogens and progesterone. In the next stage an acute inflammatory reaction is superimposed on the cystic glandular hyperplasia. Experimental evidence indicates that this reaction is determined by the level of circulating progesterone. The presence of morphologically functional corpora lutea in cases of acute endometritis in the regression phase of metoestrus suggests that a similar mechanism controls the natural disease.

If there is spontaneous recovery from the acute inflammatory reaction, presumably, due to lowered progesterone output accompanied by functional relaxation of the cervix, the expulsive process is characterised by plasma cell infiltration. This plasma cell lesion is observed at a later date in metoestrus than acute endometritis and is usually associated with regressing corpora lutea.

In the survey, chronic endometritis is preceded by a number of attacks of acute endometritis. It appears to take two forms according to the state of the cervix. Where the cervix relaxes following an attack of acute endometritis the uterus becomes small and atrophic. In some cases fibrosis of the cervix prevents discharge of fluid and the uterus remains chronically distended. Experimental evidence indicates that the changes in the latter type are largely due to chronic distension.

Appendices 1 - 7

In these appendices are shown the case histories and relevant clinical findings of all 100 cases in the series. As the same method of presentation is used for each group, notes are given at this point to explain the heading and symbols used. Pages have been made to face each other so that the details of each case may be examined more readily.

Symbols

+, ++, +++, ++++, = positive to a varying degree.

\- = negative or absent.

Where definite information was not obtained, the symbol "U" is used.

Parity. Under this heading are recorded the number of litters whelped.

Pregnancy Interval. In this column is recorded the interval between the last parturition and the examination of the uterus.

Apathy.

\- = normal behaviour.

\+ = slightly dull.

++ = very dull and apathetic.

+++ = almost moribund.

Appetite

++ = normal appetite.

\+ = eating approximately half the normal amount.

± = taking an occasional mouthful.

\- = not eating anything.

Abdominal Distension

\- = normal.

± = abdomen tense but no definite distension.

\+ = obviously distended.

++ = grossly distended.

Discharge

\- = absent

± = only a few drops on the lips of the vulva.

\+ = moderate

++ = profuse.

Thirst

\- = normal

\+ = noticeable increase in water intake.

++ = marked thirst.

Severity of Illness

\+ = symptoms of genital abnormality without general illness.

++ = showing some general upset.

+++ = seriously ill.

Appendix 1

Case Histories of Animals in Group 1 of the Cystic Hyperplasia-Pyometra Complex.

23 cases.

Case No.	Breed	Age	Stage of Cycle	Previous Oestrol Abnormalities
S 2	Terrier X	11 years	10 days Met.	+
S 3	Collie	12 years	60 days Met.	-
S 6	Airedale	7 years	Oestrus	+
S13	Collie	10 years	Anoestrus	-
S16	Terrier X	5 years	80 days Met.	+
S22	Collie	10 years	Pro-oestrus	U
S27	Dachshund	8 years	10 days Met.	+
S32	W.H.F.T.	8 years	25 days Met.	+
S33	Black Labrador	7 years	Anoestrus	-
S36	Spaniel X	10 years	40 days Met.	-
S42	Cocker Spaniel	9 years	Oestrus	+
S46	Collie	6 years	60 days Met.	+
S47	Dachshund	5 years	Pro-oestrus	-
S54	Terrier X	7 years	Anoestrus	+
S57	Scottish Terrier	4 years	30 days Met.	+
S62	Boxer	8 years	Anoestrus	-
S65	Bull Terrier	3 years	40 days Met.	+
S68	Springer Spaniel	7 years	Anoestrus	+
S74	Terrier X	4 years	70 days Met.	+

Phantom Pregnancy	Parity	Pregnancy Interval	Mating at last oestrus	Previous Genital Disease
-	1	5 years	-	-
+	2	7 years	U	-
-	-	-	+	-
+	-	-	U	-
+	-	-	U	-
U	1	8 years	-	-
-	1	3 years	U	-
-	-	-	-	-
+	-	-	-	-
-	-	-	+	-
-	1	6 years	-	-
+	-	-	-	-
-	-	-	U	-
-	-	-	U	-
-	-	-	-	-
+	1	5 years	+	-
-	-	-	-	-
-	-	-	U	-
-	-	-	U	-

Case No.	Breed	Age	Stage of Cycle	Previous Oestrol Abnormalities
S78	Cocker Spaniel	6 years	Anoestrus	U
S85	Collie	5 years	80 days Met.	+
S90	Terrier X	6 years	Anoestrus	+
S100	Black Labrador	6 years	Anoestrus	+

Phantom Pregnancy	Parity	Pregnancy Interval	Mating at last oestrus	Previous Genital Diseases
U	U	U	U	U
-	-	-	U	-
+	-	-	U	-
-	1	4 years	-	-

Appendix 2

Case Histories of Animals in Group II of the Cystic Hyperplasia-Pyometra Complex.

17 cases.

Case No.	Breed	Age	Stage of Cycle	Previous Oestrol Abnormalities
S 4	Alsatian	7 years	40 days Met.	U
S10	S.H.F.T.	8 years	50 days Met.	U
S12	Terrier X	7 years	50-60 days Met.	-
S21	Pomeranian	6 years	65 days Met.	U
S23	Collie	7 years	60-70 days Met.	-
S24	Golden Labrador	6 years	40 days Met.	+
S31	Scottish Terrier	8 years	50-60 days Met.	-
S35	Cairn Terrier	10 years	55 days Met.	+
S41	Collie	5 years	60 days Met.	+
S45	Boxer	8 years	45 days Met.	+
S53	Irish Terrier	6 years	60-70 days Met.	+
S60	S.H.F.T.	7 years	50-60 days Met.	-
S73	Springer Spaniel	7 years	50-60 days Met.	+
S80	Spaniel X	8 years	45 days Met.	-
S88	Cairn Terrier	6 years	50 days Met.	+
S93	Terrier X	5 years	65 days Met.	+
S96	Scottish Terrier	12 years	75 days Met.	+

Phantom Pregnancy	Parity	Pregnancy Interval	Mating at last oestrus	Previous Genital Disease
U	-	-	U	-
U	1	7 years	U	-
-	-	-	U	-
U	-	-	U	-
-	1	5 years	U	-
-	-	-	-	-
-	1	6 years	-	-
-	1	9 years	-	-
+	-	-	U	-
-	-	-	-	-
+	-	-	-	-
-	-	-	-	-
-	-	-	-	-
+	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-

Appendix 2

Symptomatology of Animals in Group II of the Cystic Hyperplasia-Pyometra Complex.

47 cases.

Case No.	Duration of Illness	Apathy	Appetite	Abdominal Distension
S 4	30 days	-	++	-
S10	40 days	-	++	-
S12	20 days	+	+	-
S21	10 days	+	+	-
S23	20 days	-	+	-
S24	5 days	-	++	-
S31	15 days	-	++	-
S35	40 days	-	++	-
S41	40 days	-	++	-
S45	30 days	-	++	-
S53	60 days	-	++	-
S60	15-20 days	+	+	-
S73	30 days	-	+	-
S80	20 days	-	+	-
S88	30 days	-	+	-
S93	40 days	-	+	±
S96	40-50 days	+	+	-

Discharge	Temperature	Thirst	Vomiting	Severity of Illness
+		-	-	+
++	101.4	-	-	+
+	101.8	+	Initially	++
+	101.6	+	-	++
++	101.5	+	-	+
+	102.1	-	-	+
+	101.5	-	-	+
+	102	-	-	+
+	101.1	+	-	+
+	101.7	-	-	+
+	101	-	Initially	+
++	101.8	-	-	++
+	101.6	+	-	+
+	102.1	-	-	+
+	101.6	+	-	+
+	101.3	-	Once	+
+	101.9	-	-	+

Appendix 4.

Case Histories of Animals in Group II of the Cystic Hyperplasia-Thymoma Complex.

49 animals.

Case No.	Breed	Age	Stage of Cycle	Previous Oestrous Abnormalities
S 1	Terrier X	7 years	40-45 days	-
S 5	Springer Spaniel	8 years	40-50 days	-
S 7	Collie	5 years	25-30 days Met.	-
S 8	Pekinese	10 years	28 days Met.	-
S 9	Terrier X	8 years	30-35 days	-
S14	Poodle	6 years	40-50 days	-
S15	Cocker Spaniel	7 years	15 days Met.	-
S18	Schipperke	8 years	42-49 days	-
S19	W.H.F.T.	13 years	15-20 days Met.	-
S20	Cairn Terrier	10 years	45-50 days	-
S25	Bull Terrier	5 years	21 days Met.	-
S26	Spaniel X	12 years	5 days Met.	-
S29	Alsatian	9 years	35 days	-
S30	Terrier X	6 years	70-80 days	+
S37	Black Labrador	10 years	25-30 days Met.	-
S38	West Highland Terrier	9 years	30-35 days	-
S39	Collie	13 years	7 days Met.	+
S40	Great Dane	7 years	21 days Met.	-
S43	Black Labrador	6 years	15-20 days Met.	+

Phantom Pregnancy	Parity	Pregnancy Interval	Mating at last oestrus	Previous Genital Disease[a]
-	-	-	U	U
-	-	-	U	U
-	U	-	U	U
-	2	6 years	U	U
-	-	-	U	U
-	-	-	U	U
-	-	-	-	-
-	-	-	U	U
-	3	5 years	+	? +
-	-	-	U	U
-	-	-	U	U
-	U	at least 10 years	-	+
-	-	-	U	U
-	-	-	U	U
-	-	-	+	-
-	-	-	U	-
-	1	11 years	U	+
+	-	-	-	-
-	-	-	-	-

Case No.	Breed	Age	Stage of Cycle	Previous Genital Abnormalities
S44	Terrier X	10 years	26 days Met.	+
S48	W.H.F.T.	8 years	40-45 days	-
S49	Corgi	10 years	14 days Met.	-
S50	Pekinese	10 years	20-25 days Met.	+
S51	Sealyham	6 years	30 days	-
S55	Collie	9 years	35 days	-
S56	Terrier X	8 years	20-25 days Met.	+
S58	Terrier X	11 years	15 days Met.	+
S59	Bull Terrier	4 years	30-35 days	-
S63	Cocker Spaniel	9 years	28 days Met.	+
S64	Cairn Terrier	10 years	80-90 days	+
S66	Collie	8 years	10-15 days Met.	-
S67	Terrier X	12 years	40-50 days	-
S70	Airedale	7 years	35 days	-
S71	Irish Setter	9 years	35-40 days	-
S72	Terrier X	10 years	23 days Met.	-
S75	Cocker Spaniel	8 years	28 days Met.	-
S76	Golden Labrador	11 years	35-40 days	+
S77	S.H.F.T.	6 years	40-50 days	-
S79	West Highland Terrier	9 years	20-25 days	-

Phantom Pregnancy	Parity	Pregnancy Interval	Mating at last oestrus	Previous Genital Disease
-	-	-	U	U
+	-	-	-	-
-	-	-	-	-
-	1	7 years	-	-
-	-	-	U	-
-	-	-	-	-
-	-	-	-	-
-	2	6 years	U	-
-	-	-	-	-
-	-	-	-	-
+	-	-	U	U
-	-	-	-	-
+	U	at least 7 years	-	-
-	-	-	U	-
-	-	-	-	-
-	-	-	-	-
-	1	6 years	-	-
+	-	-	-	-
-	-	-	U	-
-	-	-	U	+

Case No.	Breed	Age	Stage of Cycle	Previous Oestrol Abnormalities
S81	Alsatian	7 years	35-40 days	+
S82	Bull Terrier	9 years	14 days Met.	+
S83	Collie	8 years	30-35 days	-
S86	Terrier X	5 years	25-30 days Met.	+
S89	Corgi	6 years	40-50 days	-
S91	Bull Mastiff	8 years	15 days	+
S94	Poodle	8 years	35 days	-
S97	Collie	7 years	30-35 days	-
S98	Scottish Terrier	13 years	10-15 days	-
S99	Terrier X	10 years	25 days Met.	-

Phantom Pregnancy	Parity	Pregnancy Interval	Mating at last oestrus	Previous Genital Diseases
-	-	-	-	-
-	1	6 years	U	-
-	-	-	U	-
-	-	-	U	+
-	-	-	-	-
-	-	-	U	+
-	-	-	U	-
-	-	-	-	-
-	1	9 years	+	+
-	-	-	-	-

Appendix 5

Symptomatology of animals in Group III of the Cystic Hyperplasia-Pyometra Complex.

49 cases.

Case No.	Duration of Illness	Apathy	Appetite	Abdominal Distension
S 1	10 days	++	-	++
S 5	7 days	+	+	+
S 7	12 days	+	+	+
S 8	6 days	+	+	-
S 9	5 days	-	+	+
S14	7 days	++	±	+
S15	10 days	++	-	++
S18	4 days	+++	-	++
S19	7 days	+	+	±
S20	5 days	++	±	±
S25	20 days	+	+	+
S26	3 days	+	±	+
S29	7 days	-	+	+
S30	6 days	+++	-	±
S37	3 days	++	+	++
S38	21 days	+	+	+
S39	6 days	+	±	-
S40	4 days	+	+	+
S43	6 days	+++	-	±
S44	4 days	++	-	++
S48	14 days	+	±	+

Discharge	Temperature	Thirst	Vomition	Severity of Illness
-	102.5	++	+	+++
++	101.9	-	-	++
++	101.4	+	+	++
++	101.6	+	-	++
+	101.6	+	-	+
+	103.4	+	+	+++
-	101.9	+	++	+++
-	99.4	+	++	+++
++	101.9	+	-	++
+	103.1	+	++	+++
+	101.6	-	-	++
+	101.6	+	+	++
++	102.1	-	-	++
-	104.3	+	++	+++
+	101.6	+	-	++
Intermittent	101.9	-	-	++
++	101.9	+	-	++
+	101.6	-	-	++
++	103.0	+	+	+++
+	101.4	+	-	++
++	101.2	-	-	++

Case No.	Duration of Illness	Apathy	Appetite	Abdominal Distension
S49	3 days	+	+	±
S50	5 days	+	+	+
S51	4 days	++	±	±
S55	7 days	+	++	+
S56	8 days	+++	-	++
S58	11 days	+	+	±
S59	7 days	+++	-	++
S63	4 days	+	+	-
S64	12 days	+	++	-
S66	6 days	+++	-	++
S67	9 days	+	±	+
S70	21-28 days	+	+	+
S71	5 days	+	+	-
S72	6 days	++	±	++
S75	4 days	+	+	++
S76	9 days	+++	-	-
S77	5 days	+	+	+
S79	7 days	+	+	-
S81	9 days	+++	-	++
S82	4 days	++	±	-
S83	16 days	+	++	-

Discharge	Temperature	Thirst	Vomition	Severity of Illness
++	101.2	+	-	++
+	102.3	-	-	++
++	103.8	+	++	+++
+	101.6	-	-	++
-	101.2	+	++	+++
±	101.6	+	-	++
-	99.2	-	++	+++
++	101.3	+	-	++
++	101.9	-	-	++
-	103.6	-	++	+++
+	102.2	-	-	++
Intermittent	101.6	-	Initially	++
++	102.3	-	-	++
-	101.7	+	+	++
±	101.3	-	-	++
++	104.1	+	++	+++
+	101.8	-	+	++
++	101.2	-	Initially	++
-	103.9	+	+	+++
++	102.1	+	-	++
+	101.3	-	-	++

Case No.	Duration of Illness	Apathy	Appetite	Abdominal Distension
886	10 days	+	+	+
889	6 days	++	±	++
891	9 days	+++	-	++
894	3 days	++	±	-
897	6 days	++	+	+
898	3 days	+	±	+
899	14 days	+	±	+

Discharge	Temperature	Thirst	Vomition	Severity of Illness
±	101.6	+	-	++
Intermittent	101.9	+	+	++
Intermittent	100.1	+	++	+++
++	101.6	+	-	++
+	102.1	+	+	++
±	101.7	+	-	++
+	102.3	+	-	++

Appendix 6

Case Histories of Animals in Group IV of the Cystic Hyperplasia-Pyometra Complex.

11 animals.

Case No.	Breed	Age	Stage of Cycle	Previous Oestrol Abnormalities
S11	Collie	11 years	50-60 days	U
S17	Terrier X	10 years	65 days	U
S28	Cairn Terrier	14 years	70-80 days	+
S34	Golden Labrador	11 years	60-65 days	+
S52	Terrier X	12 years	70-80 days	+
S61	Golden Labrador	12 years	80-90 days	+
S69	Terrier X	13 years	60-70 days	+
S84	Bull Terrier	9 years	50-60 days	+
S87	Scottish Terrier	15 years	70-80 days	+
S92	Cairn Terrier	12 years	65 days	+
S95	Great Dane	11 years	60-70 days	+

Phantom Pregnancy	Parity	Pregnancy Interval	Mating at last oestrus	Previous Genital Disease
U	U	U	U	U
U	U	U	U	U
-	-	-	-	+
-	-	-	-	+
-	-	-	-	+
+	-	-	-	+
-	2	6 years	-	+
-	-	-	U	+
-	2	9 years	-	+
-	-	-	-	+
+	-	-	+	+

Appendix 7

Symptomatology of animals in Group IV of the Cystic Hyperplasia-Pyometra Complex.

11 animals.

Case No.	Duration of Illness	Apathy	Appetite	Abdominal Distension
S11	50 days	-	++	-
S17	60 days	++	±	++
S28	30 days	+++	-	++
S34	50 days	+++	-	++
S52	70 days	-	++	-
S61	70-80 days	+	+	-
S69	60-70 days	++	-	++
S84	20-30 days	+++	-	++
S87	60-70 days	-	++	±
S92	50-60 days	+++	-	++
S95	40-50 days	++	±	++

Discharge	Temperature	Thirst	Vomition	Severity of Illness
++	101.7	-	-	+
±	102.5	++	+	++
-	100.3	+	+	+++
-	99.9	+	++	+++
++	101.9	-	-	+
++	102.1	-	-	++
-	102.2	+	+	+++
-	103.6	+	+	+++
++	101.2	+	-	+
-	100.7	++	++	+++
±	102.3	+	+	++

Appendix 8

Total and Differential Leucocyte Counts in 17 cases exhibiting Type 2 lesion of the endometrium.

Case	Total per c.mm.	Non-Lob. Neutro-phil	Lob. Neutro-phil	Baso-phil	Eosino-phil	Lympho-cyte	Mono-cyte
S 4	13,700	0	67	0	7	23	3
S10	10,600	0	62.5	0.5	1.5	30.5	5
S12	12,000	0.5	70.5	0	2	26	1
S21	14,600	1	75.5	0	3.5	18	2
S23	15,100	1.5	71.5	0	2	23	2
S24	12,200	0	74	0.5	1.5	21	3
S31	9,900	0	76	0	3	19.5	1.5
S35	11,400	0.5	67	0	3.5	27	2
S41	14,700	0.5	71.5	0	2	24	2
S45	12,200	0	65.5	0	4	25.5	5
S53	13,200	1.5	68.5	0	3	23.5	3.5
S60	14,100	1	70	0	2.5	24	2.5
S73	10,200	0	68.5	0	1	24.5	6
S80	10,900	0	76.5	0	2.5	28	2
S88	11,700	0	67	0	3	27.5	2.5
S93	12,900	0.5	67	0	3.5	26	3
S96	13,500	0.5	66	0	2	29.5	2

Appendix 9

Total and Differential Leucocyte Counts in 49 cases of Acute Endometritis.

Case	Total per c.mm.	Non-Lob. Neutro-phil	Lob. Neutro-phil	Baso-phil	Eosino-phil	Lympho-cyte	Mono-cyte
S 1	50,100	6.5	72.5	0	1.5	15.5	4
S 5	21,850	2	74	0	3.5	19	1.5
S 7	43,450	11.5	78	0	1.5	8	1
S 8	31,350	3	61.5	0.5	7	23	5
S 9	25,100	1.5	73	0	1.5	20	4
S14	43,100	9	74	0	3	12	2
S15	61,250	13	79	0	0	6.5	1.5
S18	145,000	23.5	69.5	0	0	5.5	1.5
S19	19100	1	63.5	0	6	25	4.5
S20	73,000	31	63.5	0	0	4.5	1
S25	25,100	3.5	69	0.5	2	21	4
S26	39,250	16.5	71	0	1	10.5	1
S29	22,300	0	70	0	3	24	3
S30	86,200	6.5	77	0	2	9	5.5
S37	44,800	19.5	69.5	0	0	7.5	3.5
S38	29,400	5	74	0	1	16.5	3.5
S39	38,600	3.5	84.5	0	1.5	8.5	2
S40	29,300	6	79	0	0.5	11	3.5
S43	84,500	8.5	81	0	1	7	2.5
S44	46,100	1.5	87.5	0	2.5	5.5	3

Case	Total per c.mm.	Non-Lob. Neutro-phil	Lob. Neutro-phil	Baso-phil	Eosino-phil	Lympho-cyte	Mono-cyte
S48	30,600	0.5	87	0	1	9.5	2
S49	22,350	0	76	0	3.5	17.5	3
S50	25,300	1.5	70.5	0	3.5	20	4.5
S51	52,000	3.5	84.5	0	2	7	3
S55	19,350	3.5	68.5	0	5	19	4
S56	59,700	7	83	0	2.5	6.5	1
S58	21,400	0	69.5	0	1.5	25	4
S59	125,200	34	56	0	1	4.5	4.5
S63	37,000	13.5	65.5	0	2	16	3
S64	24,550	0.5	83	0	1.5	14	1
S66	50,200	20	66	0	0	11.5	2.5
S67	39,050	3	88	0	0.5	6.5	2
S70	20,850	0	80	0	5	15	0
S71	23,600	5	71.5	0	1.5	18.5	3.5
S72	41,900	16	83.5	0	0.5	8.5	1.5
S75	32,700	10	80	0	0	8	2
S76	94,200	23	66	0	0	10.5	0.5
S77	27,300	0.5	83	0	3	13	0.5
S79	25,850	0	81	0	1.5	16	1.5
S81	62,500	7.5	83.5	0	1	7	1

Case	Total per c.mm.	Non-Lob. Neutro-phil	Lob. Neutro-phil	Baso-phil	Eosino-phil	Lympho-cyte	Mono-cyte
S82	32,050	1.5	86	0	0	10.5	2
S83	18,950	3	76	0	2	18	1
S86	27,200	1	82	0	1.5	12	3.5
S89	63,050	20	72	0	0.5	4.5	3
S91	112,500	22.5	73	0	0.5	4	0
S94	28,400	2	76	0.5	2.5	16.5	2.5
S97	37,200	3.5	81.5	0	1	12	2
S98	21,000	0	79	0	2	17.5	1.5
S99	29,350	4	74	0	2	16.5	3.5

Appendix 10

Total and Differential Leucocyte Counts in Animals in Group IV of the Cystic Hyperplasia-Pyometra Complex.

11 animals.

Case	Total per c.mm.	Non-Lob. Neutro-phil	Lob. Neutro-phil	Eosino-phil	Lympho-cyte	Mono-cyte	Abnormal Mono-cyte
S11	16,500	0	67	2	26	3	2
S17	31,000	1	66	1.5	22	2	7.5
S28	62,300	3.5	77.5	1.5	12	2	3.5
S34	39,700	1.5	75	3	14.5	1	5
S52	19,300	0	67.5	2	25.5	5	0
S61	21,450	0	71	3.5	18.5	7	0
S69	33,400	2.5	76	4	12.5	4	1
S84	67,900	4	81.5	0.5	5	2	7
S87	17,500	0	68.5	4	21	4.5	2
S92	52,100	2.5	80.5	2.5	5.5	3	6
S95	41,750	1.5	74	1.5	17	3.5	2.5

Appendix 11

Haematology of Experimental Animals.

Case No.	Day of Experiment	W.B.C. per c.mm.	Non-Lob. Neutro-phil	Lob. Neutro-phil	Baso-phil
D1	1	5,900	0	63	0
	3	15,800	1	81	0
	5	17,500	1	85	0
	7	21,450	2.5	84	0
	10	27,400	2	83.5	0
D2	1	9,600	1	64	0
	3	13,100	1.5	69	0
	5	17,300	1.5	79	0
	7	24,600	5	81.5	0
	10	29,200	2	82	0
D3	1	8,700	0.5	67	0
	4	12,600	1	73	0
	7	14,100	0.5	75.5	0
	10	23,700	2.5	77	0
	13	29,800	3.5	78.5	0
	16	35,600	6	83	0
	19	42,700	8	81.5	0
	21	42,000	5	82.5	0

Eosino-phil	Lympho-cyte	Mono-cyte	R.B.C. 10^3 per c.mm.	Haemoglobin g/100ml	E.S.R.
5	27	5	5,900	15.1	0
1.5	14	2.5	5,800	14.9	0
0.5	12.5	1	5,900	15	0
1	11	1.5	5,200	13.8	0
1	12.5	1	5,100	13.6	12
2.5	28	4.5	5,500	13.9	1
3	21.5	5	5,600	14	3
2	15	2.5	5,400	13.7	19
1.5	10	2	5,400	13.6	26
1	14	1	5,100	13.1	32
3	26	3.5	5,950	14.5	0
2.5	21.5	2	5,900	14.4	1
1	21.5	1.5	5,700	14.3	7
1.5	18	1	5,800	14.4	15
0	17	1	4,950	13.6	20
1	9.5	0.5	4,225	13.1	29
0.5	10	0	4,250	13	42
0	12	0.5	4,125	12.8	45

Case No.	Day of Experiment	W.B.C. per c.mm.	Non-Lob. Neutro-phil	Lob. Neutro-phil	Baso-phil
D 4	1	8,600	0.5	65	0
	4	12,200	0	68	0
	7	15,900	1.5	71.5	0
	10	21,300	1	76.5	0
	13	28,600	2.5	79	0
	16	34,000	2	82	0
	19	43,200	2.5	84	0
	22	51,600	4.5	86.5	0
	24	63,500	3.5	91	0
	26	4,100	0	49.5	0
D5	1	9,600	0	73.5	0
	4	11,200	0	74	0
	7	15,700	0.5	75.5	0
	10	18,100	1	77	0
	13	23,200	1	78	0
	16	29,300	0.5	79.5	0
	19	37,600	2	82	0
	22	39,000	3.5	84	0
	25	41,200	3	85	1
	26	24,300	0.5	70.5	0

Eosino-phil	Lympho-cyte	Mono-cyte	R.B.C. 10^3 per c.mm.	Haemoglobin g/100ml	E.S.R.
4.5	26.5	3.5	6,100	14.2	0
3	25	4	6,200	14.1	0
3.5	21	2.5	5,900	13.7	2
2.5	17	3	5,750	13.5	9
1	15.5	2	5,625	13.3	17
1.5	12.5	2	5,400	12.8	24
2.5	10.5	0.5	4,900	11.7	31
1.5	6	1.5	4,600	11.1	30
1.5	2.5	1.5	4,300	10.2	37
2.5	41	7	3,900	9.8	39
4	17.5	5	5,600	13.7	1
3	19.5	3.5	5,800	13.9	1
3.5	16.5	4	5,700	13.8	7
2	18	2	5,750	13.8	10
2.5	16	2.5	5,600	13.6	14
1.5	17	1.5	5,650	13.7	16
1	14	1	5,500	13.5	17
0.5	11.5	0.5	5,500	13.4	19
1.5	8.5	1	5,300	13.1	27
2.5	22	4.5	5,200	13	31

Case No.	Day of Experiment	W.B.C. per c.mm.	Non-Lob. Neutro-phil	Lob. Neutro-phil	Baso-phil
D7	1	9,700	0.5	68	0
	3	12,900	0	71	0
	5	15,600	1	71	0
	7	19,200	1.5	75	0
	10	20,700	1.5	80.5	0
D9	1	7,900	0	72	0
	4	11,400	0	75	0.5
	7	13,600	1	76	0
	10	14,200	0.5	79	0
	13	17,900	1	78.5	0
	16	21,400	1.5	80	0
	19	23,600	1	81.5	0
	23	26,200	0.5	81	0
	26	26,700	1	82	0
	30	27,300	1	83	0
D10	1	9,200	0	73	0
	4	11,300	0.5	71	0
	7	12,100	0	71.5	0
	10	15,600	1	72	0
	13	19,200	1.5	77	0

Neutro-phil	Lympho-cyte	Mono-cyte	R.B.C. 10^6 per c.mm.	Hemoglobin g/100ml	E.S.R.
3.5	22	4	6,100	14.2	0
2.5	21.5	5	6,300	14.4	1
3	21	4	6,150	14.2	3
1.5	10.5	3.5	6,050	14.1	5
3	19	2	6,100	14.2	10
2.5	20.5	5	6,000	14.1	1
2	20	2.5	6,200	14.0	7
3.5	15.5	4	6,100	14.1	10
1	16	3.5	5,900	13.9	15
1.5	16.5	2.5	5,800	13.6	23
3	14.5	1	5,600	13.1	27
2.5	13.5	1.5	5,500	12.7	34
2	16	0.5	4,700	11.1	39
1	15	1	4,100	10.2	42
1.5	13	1.5	3,400	8.7	44
1.5	21.5	4	5,700	13.2	0
3	22	3.5	5,900	13.5	0
3.5	21.5	3.5	5,600	13.2	1
2.5	22.5	2	5,500	13.1	9
2	17.5	2	5,500	13.2	11

Case No.	Day of Experiment	W.B.C. per c.mm.	Non-Lob. Neutro-phil	Lob. Neutro-phil	Baso-phil
D10	16	24,700	1	79	0
	19	27,600	2.5	81	0
	23	30,300	3	84.5	0
	27	31,200	1.5	87	0
	30	35,600	0.5	86	0
	33	34,200	0	82	0
	36	16,100	0	84	0
	38	4,200	0	42	0
D12	1	10,500	0.5	72	0
	3	12,200	1	73.5	0
	5	25,600	3.5	79	0
	7	46,100	4	86.5	0
	9	45,900	1.5	88	0
	11	42,800	0	87.5	0
D14	1	9,600	0.5	72	0
	3	10,500	0	73	0
	5	9,700	0	74.5	0
	7	9,300	0.5	71.5	0
	10	8,900	1	74	0

Eosino-phil	Lympho-cyte	Mono-cyte	R.B.C. 10^3 per c.mm.	Haemoglobin g/100ml	E.S.R.
3	15.5	1.5	5,300	13	14
2	13.5	1	5,100	12.8	19
1.5	10	1	4,700	12.1	22
1	10	0.5	4,300	11.4	29
2	11	0.5	4,000	10.7	33
1.5	15.5	1	3,700	10.2	31
0.5	33	2.5	3,200	9.6	32
0	52	6	2,800	9.1	39
2.5	21.5	3.5	5,600	13.1	0
2	20.5	3	5,700	13.1	9
1.5	14.5	1.5	5,600	13.0	22
1.5	7	1	5,500	12.9	31
2	8	0.5	5,500	13	38
2.5	9	1	5,400	12.9	45
2	20.5	5	5,100	12.7	0
3	20.5	3.5	5,300	12.9	1
1.5	20	4	5,200	12.8	0
2	23.5	2.5	5,300	12.8	0
4	18	3	5,200	12.7	1

Cage No.	Day of Experiment	W.B.C. per c.mm.	Non-Lob. Neutro-phil	Lob. Neutro-phil	Baso-phil
D18	1	10,900	0.5	68.5	0
	4	15,300	1	72	0
	7	21,200	1.5	75	0
	10	23,600	1.5	76.5	0
	13	26,100	1	78	0
	16	15,900	0	72.5	0
	18	11,300	0	71	0
	20	11,500	0	70.5	0
D20	1	9,600	0.5	71	0
	4	10,700	0	74	0
	7	11,100	1	73.5	0
	10	10,300	0	69	0
	13	9,700	0.5	71.5	0
	16	10,800	0	73	0
	18	10,100	0	72.5	0
	20	9,900	0.5	70.5	0
D23	1	10,800	0	68.5	0
	4	13,700	1	71	0
	7	17,900	0.5	72.5	0

Neutrophil	Lymphocyte	Monocyte	R.B.C. 10^3 per c.mm.	Haemoglobin g/100ml	E.S.R.
3	23	5	5,900	13.5	0
1.5	21.5	4	5,900	13.5	4
2	18	3.5	5,800	13.5	11
2.5	17	2.5	5,900	13.6	19
2.5	16.5	2	5,700	13.5	25
3	21.5	3	5,700	13.4	14
1.5	24.5	3	5,600	13.4	3
2.5	24.5	2.5	5,700	13.4	1
1	21.5	6	6,100	14	0
1.5	21	3.5	6,000	14	1
1.5	19	5	6,100	14.1	0
0.5	26.5	4	5,900	14	0
1	24.5	2.5	6,000	14.1	0
2	21.5	3.5	6,200	14.2	0
1.5	22	4	6,100	14.1	0
1	24.5	3.5	6,200	14.1	0
2.5	25	4	4,900	12.1	0
2	21.5	4.5	5,000	12.1	4
1.5	22.5	3	5,200	12.3	7

Case No.	Day of Experiment	W.B.C. per c.mm.	Non-Lob. Neutro-phil	Lob. Neutro-phil	Baso-phil
D25	10	21,300	1	75	0
	14	23,700	2	75.5	0
	17	14,300	0.5	74	0
	20	10,200	0	71.5	0
	23	10,700	0	71	0
	27	9,800	0.5	70.5	0
	30	10,100	0.5	72	0
D28	1	11,600	0.5	71.5	0
	4	14,900	1.5	74	0
	7	21,300	2	75.5	0
	10	27,600	1.5	79	0
	15	16,100	0.5	73	0
	20	10,200	0.5	71	0
	25	11,900	0	72.5	0
	30	10,600	1	69	0
	35	9,300	0	71	0
	40	10,500	0	70.5	0
	45	11,100	0.5	74	0
	50	10,700	0.5	70	0

Eosino-phil	Lympho-cyte	Mono-cyte	R.B.C. 10^3 per c.mm.	Haemoglobin g/100ml	E.S.R.
2	19.5	2.5	5,100	12.2	16
3	18.5	1	4,900	12.2	20
1.5	22.5	1.5	5,100	12.2	12
1.5	25.5	1.5	5,000	12.1	6
2	24.5	2.5	5,200	12.1	2
1.0	25	3	5,200	12.2	2
2.5	22.5	2.5	5,100	12.2	0
3.5	21.5	3	5,100	12.5	0
2.5	20	2	5,400	12.7	6
3	18.5	1	5,200	12.6	9
2.5	16	1	5,000	12.6	17
2	22	2.5	5,400	12.8	16
2	23.5	3	5,300	12.7	2
1.5	23	3	5,100	12.6	1
3	24.5	2.5	5,500	12.9	1
4	23	2	5,300	12.7	2
1.5	24.5	3.5	5,700	12.8	0
4	18.5	3	5,400	12.6	0
2.5	24.5	2.5	5,600	12.7	1

Case No.	Day of Experiment	W.B.C. per c.mm.	Non-Lob. Neutro-phil	Lob. Neutro-phil	Baso-phil
D30	1	11,100	0.5	74	0
	5	17,600	1.5	76.5	0
	10	22,300	2	82.5	0
	15	26,100	3	84	0
	20	14,200	0.5	76.5	0
	25	10,100	1	72	0
	30	10,700	0.5	71.5	0
	35	11,300	0	72	0
	40	10,200	0	69.5	0
	45	9,800	1.5	70.0	0
	50	9,200	0.5	71	0
	55	11,700	0	71.5	0
	60	10,100	0	74	0
	65	10,900	1	77	0
	70	9,800	0	74.5	0
D32	1	10,200	0.5	68.5	0
	4	13,700	1.5	68	0
	7	19,200	1.5	71.5	0
	10	23,400	2.5	76.5	0
	14	27,300	2	79	0

Eosino-phil	Lympho-cyte	Mono-cyte	R.B.C. 10^3 per c.mm.	Haemoglobin g/100ml	E.S.R.
1.5	19	5	5,300	12.6	0
2	17.5	2.5	5,200	12.5	1
1	13	1.5	5,300	12.5	17
1.5	10	1.5	5,000	12.2	21
0.5	20	2.5	5,000	12.3	0
2.5	21.5	3	5,100	12.3	2
3	21.5	3.5	5,000	12.2	0
2	22	4	5,200	12.4	0
2.5	25.5	2.5	5,300	12.4	0
1.5	23.5	3.5	5,200	12.3	0
1	26	1.5	5,200	12.3	1
2	24	2.5	5,300	12.5	0
2.5	20	3.5	5,400	12.5	2
3.0	17	3	5,500	12.6	0
2.0	21.5	2	5,600	12.8	0
3.5	23.5	4	6,100	13.7	0
2	25.5	3	6,200	13.7	4
3	21.5	2.5	6,300	13.8	9
1.5	18	1.5	6,100	13.6	16
2	16	1	6,000	13.4	22

Cage No.	Day of Experiment	W.B.C. per c.mm.	Non-Lob. Neutro-phil	Lob. Neutro-phil	Baso-phil
D32	17	16,700	0.5	74.0	0
	20	12,100	0	71	0.5
	23	10,600	0	69.5	0
	26	10,900	0.5	68.5	0
	29	11,200	0	71.5	0
	32	12,700	0.5	75	0
	36	18,600	1.5	76.5	0
	41	21,200	1.5	76	0
	44	23,500	1.0	79.5	0
	48	15,400	0.5	74	0
	52	11,200	0.5	70.5	0.5
	55	10,900	0	71	0
	56	10,400	0	68	0
	60	11,100	0.5	67.5	0
D34	1	9,200	0.5	71	0
	6	16,700	1	75.5	0
	12	25,200	2.5	79.5	0
	18	17,100	1	80	0
	24	10,900	0	72.5	0
	30	10,100	0	69	0

Eosino-phil	Lympho-cyte	Mono-cyte	W.B.C. 10^3 per c.mm.	Haemoglobin g/100ml	E.S.R.
1	23	1.5	6,100	13.6	6
1.5	25	2	6,200	13.8	2
2	26	2.5	6,100	13.7	1
3	26	2	6,000	13.5	0
2.5	23.5	2.5	5,900	13.3	0
1	21.5	2	5,900	13.2	3
1.5	19	1.5	5,800	13	8
1	20.5	1	5,800	13.1	19
2.5	16	1	5,500	12.6	16
3	21	1.5	5,500	12.5	4
3	23	2.5	5,400	12.3	2
1.5	25	2.5	5,500	12.6	0
2	27	3	5,500	12.6	1
2.5	27	2.5	5,500	12.5	0
2.5	22	4	6,500	14.2	0
3.5	16.5	3.5	6,300	13.8	5
3.0	12.5	2.5	6,400	13.9	19
1.5	15.5	2.0	6,400	13.9	6
2.5	21.5	3.5	6,500	14.1	2
1.5	25.5	4	6,400	13.9	1

Case No.	Day of Experiment	W.B.C. per c.mm.	Non-Lob. Neutro-phil	Lob. Neutro-phil	Baso-phil
D34	36	17,100	0.5	76.5	0
	42	24,600	2	79	0
	48	14,200	0.5	74.5	0
	55	10,100	0	69	0
	61	11,200	0.5	68.5	0
	68	15,600	1.5	75.5	0
	74	19,100	2.5	80.5	0
	80	11,300	0.5	71.5	0
	85	10,000	0.5	70.5	0
	96	13,200	0.5	72	0
	103	17,300	1.5	76.5	0
	109	12,100	0	72	0
	115	10,700	0	70	0
	120	9,600	0.5	69.5	0
D36	1	10,800	0.5	73.5	0
	6	19,200	2.5	78.5	0
	12	29,600	3	83	1.0
	18	15,300	0.5	77.5	0
	24	11,200	0	76.5	0
	30	10,700	0	71	0

Eosino-phil	Lympho-cyte	Mono-cyte	R.B.C. 10^3 per c.mm.	Haemoglobin g/100ml	E.S.R.
2	18.5	2.5	6,400	14	9
3.5	14.5	1	6,300	13.8	16
2	21	2	6,300	13.7	4
1.5	27	2.5	6,200	13.6	0
1	27	3	6,200	13.5	0
2	19	2	6,000	13.1	22
2.5	13	1.5	5,600	12.4	25
2	23.5	2.5	5,500	12.3	11
2.5	23.5	3	5,400	12.1	2
2	22	3.5	5,000	11.5	23
2	17.5	2.5	4,600	10.8	31
3.5	22	2.5	4,200	10.3	14
3	23	4	4,100	10.1	7
2	24.5	3.5	3,900	9.7	3
3	16	7	5,700	13	1
1	14.5	3.5	5,800	13.1	16
2	9.5	1.5	5,600	12.8	19
0.5	19	2.5	5,700	12.9	7
2.5	16	5	5,800	13	1
3	21.5	4.5	5,600	12.9	0

Case No.	Day of Experiment	W.B.C. per c.mm.	Non-Lob. Neutro-phil	Lob. Neutro-phil	Baso-phil
D36	36	17,900	0.5	77.5	0
	43	24,700	1.5	82.5	1.5
	49	12,200	0.5	71	0
	56	11,600	0	73	0
	62	13,600	0.5	74.5	0
	68	17,100	2	78.5	0
	73	21,300	1.5	81	0
	81	10,700	0.5	73.5	0
	87	10,100	0	70.5	0
	95	14,700	0	75.5	0
	102	18,600	2	77	0
	109	12,800	0	72	0.5
	116	10,700	0	71	0
	122	12,300	1.5	72.5	0
	130	17,300	1.5	79	0
	137	12,400	0.5	71.5	0
	145	11,000	0	71	0
	153	12,100	0	72.5	0
	160	18,700	0.5	78	0
	168	10,700	0.5	72	0
	174	11,100	0	71.5	0
	180	10,200	0	72.5	0

Eosino-phil	Lympho-cyte	Mono-cyte	R.B.C. 10^3 per c.mm.	Haemoglobin g/100ml	E.S.R.
1.5	18	2.5	5,500	12.8	17
2.5	10.5	1.5	5,600	12.8	22
0.5	26.5	1.5	5,400	12.5	9
1	22.5	3.5	5,400	12.4	2
1.5	21	2.5	5,300	12.2	2
2.5	16	1	5,400	12.3	21
3.5	13.5	0.5	5,300	12.3	25
1.5	22.5	2.0	5,200	12	1
2	24.5	3	5,200	12.1	2
3	17.5	4	5,300	12.1	16
2.5	16.5	2	5,000	11.8	27
3	22	2.5	5,100	11.9	10
2	24	3	5,200	11.9	2
1.5	21.5	3	4,900	11.5	7
1.5	16	2	4,800	11.3	26
2.5	23	2.5	4,600	11	10
1	24	4	4,500	10.9	2
1.5	22.5	3.5	4,200	10.4	2
2	18	1.5	4,000	10	29
1.5	23.5	2.5	4,000	9.9	22
2.5	23	3	3,500	9.0	18
1.5	23	3	3,400	8.9	8

Case No.	Day of Experiment	W.B.C. per c.mm.	Non-Lob. Neutro-phil	Lob. Neutro-phil	Eosino-phil
140	1	11,800	0	73	0
	6	17,600	1.5	76.5	0
	12	27,300	2	81	0.5
	18	14,700	0.5	75	0
	23	10,800	0	72.5	0
	30	11,100	0.5	71	0
	37	18,200	1	77	0
	43	25,600	2.5	83	0
	49	11,700	0.5	73.5	0
	55	10,400	0.5	74	0
	62	12,200	1	75.5	0
	68	17,000	1.5	79.5	0.5
	73	22,600	1.5	81.5	0
	81	11,300	0	73	0
	88	11,600	0.5	72	0
	95	16,100	0.5	78.5	0
	102	19,300	0.5	81	0
	109	11,700	1	72.5	0
	116	10,900	0	70.5	0
	123	12,300	0.5	72.5	0
	130	19,700	1	80.5	0

Eosino-phil	Lympho-cyte	Mono-cyte	R.B.C. 10^3 per c.mm.	Haemoglobin g/100ml	E.S.R.
2.5	20.5	4	6,000	13.8	1
3	14.5	4.5	6,100	13.8	2
2	12	2.5	6,100	13.9	16
1.5	20.5	2.5	6,000	13.8	5
2	22.5	3	6,200	13.9	2
2.5	23	3	6,100	13.8	1
2.5	16.5	3	6,000	13.7	17
2	10	2.5	6,000	13.7	21
3	20.5	2.5	6,100	13.8	11
1.5	21	3	6,000	13.6	2
1.5	19	3	5,900	13.5	3
2	14.5	2	6,000	13.5	19
2	14	1	6,100	13.6	20
2.5	22	2.5	6,000	13.5	7
2	22.5	3	6,000	13.5	1
3	16	2	6,100	13.5	14
2.5	14.5	1.5	6,000	13.4	25
2.5	22	2	6,000	13.4	6
1.5	25	3	5,800	13	2
1	22	4	5,900	13	11
1.5	15	2	5,800	12.8	29

Case No.	Day of Experiment	W.B.C. per c.mm.	Non-Lob. Neutro-phil	Lob. Neutro-phil	Baso-phil
D40	136	13,100	0.5	74	0
	143	10,300	0.5	71	0
	150	11,200	0	71.5	0
	154	13,600	0.5	74.5	0
	157	17,300	1.5	78.5	0
	161	18,900	1	80.5	0
	164	15,200	1	76.5	0
	168	10,900	0.5	71	0
	171	11,400	0.5	70.5	0
	173	15,100	1	74.5	0
	176	17,300	1.5	77	0
	178	18,000	1	78.5	0
	180	19,100	1.5	79.5	0.5
D43	1	9,100	0.5	71.5	0
	4	12,900	0.5	73	0
	7	16,700	1	77.5	0
	10	19,200	2	79.5	0
	13	22,300	1.5	82	0
	16	15,700	0.5	76	0
	19	11,200	0.5	71	0

Eosino-phil	Lympho-cyte	Mono-cyte	R.B.C. 10^3 per c.mm.	Haemoglobin g/100ml	E.S.R.
2.5	20.5	2.5	5,700	12.7	13
1.5	24	3	5,600	12.4	6
2	24	2.5	5,400	11.9	3
2.5	20	2.5	5,200	11.6	9
3	15	2	5,200	11.5	16
2.5	15	1	5,000	11	27
2	19	1.5	4,700	10.7	25
2.5	24	2.0	4,600	10.5	6
2	24	3	4,500	10.4	2
2.5	18.5	3.5	4,400	10.2	7
1.5	17	3	4,400	10.1	11
2	16	2.5	4,300	10	13
2	14	2.5	4,200	9.9	16
3.5	20	4.5	5,200	12	0
2.5	20	4	5,100	11.9	3
3	16	2.5	5,300	12.1	8
1.5	15.5	1.5	5,100	12.1	16
2	13.5	1	5,000	12	20
2.5	19	2	5,200	12.2	7
1.5	24.5	2.5	5,300	12.3	2

Case No.	Day of Experiment	W.B.C. per c.mm.	Non-Lob. Neutro-phil	Lob. Neutro-phil	Baso-phil
D43	22	10,600	0	72.5	0
	26	11,100	0.5	71	0
	30	10,800	0.5	70.5	0
D44	1	10,900	0	68.5	0
	6	15,800	0.5	73.5	0
	12	22,900	3	81	0
	16	17,600	1.5	76.5	0
	22	11,200	0.5	71	0
	30	10,700	0	72	0
	36	16,800	0.5	74.5	0
	41	21,700	2	82	0.5
	47	12,200	0.5	72	0
	51	11,600	0.5	70.5	0
	55	12,100	0	68.5	0
	58	10,800	0.5	70	0
	60	10,000	0	70.5	0
D46	1	11,200	0	70	0
	6	16,000	0.5	76.5	0
	12	25,700	2.5	82.5	0

Eosino-phil	Lympho-cyte	Mono-cyte	R.B.C. 10^3 per c.mm.	Haemoglobin g/100ml	E.S.R.
2	23	2.5	5,400	12.4	1
2.5	23	3	5,300	12.4	0
2	23	4	5,400	12.4	1
1.5	26	4	5,000	11.9	0
1	22	3	5,100	12	8
0.5	14	1.5	4,900	11.7	16
2	18	2	5,000	11.9	2
1.5	23.5	3.5	5,000	11.9	1
2	23	3	4,900	11.8	0
1	22	2	4,900	11.7	9
1.5	13	1	4,800	11.6	21
2	23	2.5	4,900	11.7	7
2.5	23	3.5	4,800	11.5	2
1.5	27	3	4,700	11.3	1
2.5	24.5	2.5	4,700	11.4	0
1	25.5	3	4,700	11.3	1
3	22.5	4.5	5,100	11.6	0
2.5	17.5	3	5,200	11.7	10
2	11	2	5,000	11.6	17

Case No.	Day of Experiment	W.B.C. per c.mm.	Non-Lob. Neutro-phil	Lob. Neutro-phil	Baso-phil
D46	18	12,300	0.5	72	0
	23	10,900	0	70.5	0
	30	11,400	0.5	71	0
	36	17,600	1	77	0
	42	24,900	3	82	0.5
	49	13,800	1	73.5	0
	55	10,200	0.5	70.5	0
	61	10,700	0.5	70	0
	67	19,200	1	78	0
	73	21,600	1	81.5	0
	76	13,400	0	73	0
	79	11,200	0	71.5	0
	82	11,700	0.5	71.5	0
	86	14,300	1	73.5	0
	90	18,700	1	77.5	0
	94	21,200	1.5	80	0
	98	24,200	2	81.5	0
	102	22,000	0.5	80.5	0
	105	15,300	0.5	76	0
	108	12,100	0	72	0
	110	14,700	0.5	75	0

Eosino-phil	Lympho-cyte	Mono-cyte	R.B.C. 10^3 per c.mm.	Haemoglobin g/100ml	E.S.R.
3	21	3.5	5,000	11.5	6
3.5	22	4	5,100	11.6	2
2	23.5	3	5,000	11.5	1
2.5	17	2.5	5,000	11.4	12
2	11.5	1	4,900	11.2	18
1.5	22.5	1.5	4,900	11.2	5
2	24.5	2.5	5,000	11.3	0
3	23.5	3	4,900	11.2	3
1.5	17.5	2	4,800	11	15
2	14	1.5	4,800	11	22
1.5	23	2.5	4,700	10.8	4
2.5	23	3	4,800	10.9	2
2	22.5	3.5	4,700	10.8	1
3	19.5	3	4,700	10.8	4
2.5	17.5	1	4,600	10.7	8
2.5	15	1	4,500	10.6	11
1.5	14	1	4,500	10.6	21
2	16.5	0.5	4,400	10.4	25
2.5	19.5	1.5	4,200	10.2	11
2	24.5	1.5	4,100	10.1	6
1.5	21	2	4,000	10	9

Case No.	Day of Experiment	W.B.C. per c.mm.	Non-Lob. Neutro-phil	Lob. Neutro-phil	Baso-phil
D46	113	23,400	1	80.5	0
	116	35,000	3.5	86.5	0
	120	33,900	3	85	0
D51	1	10,700	0.5	67.5	0
	6	15,800	0.5	73.5	0
	12	24,300	2	79.5	0.5
	18	11,900	1	71	0
	24	10,800	0	71.5	0
	30	11,000	0.5	70	0
	37	18,100	1	76.5	0
	43	23,700	1.5	82.5	0
	48	12,800	0.5	73	0
	54	11,200	0	71.5	0
	61	12,700	0	72	0
	68	19,100	0.5	78.5	0
	73	22,000	1	81.5	0
	81	11,000	0.5	71.5	0
	88	10,400	0.5	70.5	0
	95	16,700	1	76	0
	102	21,600	2	80.5	0.5

Eosino-phil	Lympho-cyte	Mono-cyte	R.B.C. 10^3 per c.mm.	Haemoglobin g/100ml	E.S.R.
2.5	15	1	4,100	10.1	21
2	7.5	0.5	4,000	10.0	26
1.5	9.5	1	3,900	10.0	28
2.5	23.5	6	5,800	12.9	1
3	18.5	4.5	5,900	13	8
4	11.5	2.5	5,900	12.9	17
3	21.5	3.5	5,800	12.8	2
3.5	22	3	5,800	12.8	1
2.5	23.5	3.5	5,700	12.6	0
2.5	18	2	5,600	12.5	13
3.5	10.5	2	5,700	12.6	18
3	21	2.5	5,700	12.5	5
2	23.5	3	5,600	12.4	1
2.5	23	2.5	5,700	12.5	3
2	17.5	1.5	5,600	12.4	17
3.5	13	1	5,500	12.2	22
1.5	24	2.5	5,400	12.1	3
2	24	3	5,400	12	1
2.5	19	1.5	5,200	11.7	14
3	13	1	5,000	11.3	21

Case No.	Day of Experiment	W.B.C. per c.mm.	Non-Lob. Neutro-phil	Lob. Neutro-phil	Baso-phil
D51	107	12,100	0.5	71	0
	110	13,700	0.5	74.5	0
	114	17,200	1	77	0
	118	21,100	1	81	0
	120	25,600	2	83.5	0
	124	24,200	1.5	83	0
	126	13,700	0.5	74	0
	129	10,900	0	71	0
	132	11,100	0.5	70.5	0
	135	10,600	0	71	0
D56	1	11,100	0.5	71.5	0.5
	6	16,700	1	76	0
	13	25,900	3.5	84.0	0.5
	20	11,600	0.5	72.5	0
	27	10,800	0	71.5	0
	35	18,200	2.5	77.5	0
	42	25,000	3.5	83.5	0
	48	12,200	0.5	74.5	0
	55	10,700	0.5	71	0
	61	11,200	0	70	0

Eosino-phil	Lympho-cyte	Mono-cyte	R.B.C. 10^2 per c.mm.	Haemoglobin g/100ml	E.S.R.
1.5	24.5	2.5	4,700	10.7	7
2	20.5	2.5	4,500	10.4	9
2.5	17.5	2	4,300	10.1	12
2.5	13.5	2	4,300	10	20
3	10	1.5	4,200	9.8	23
2	12	1.5	4,100	9.7	25
2.5	21.0	2	4,100	9.6	7
2	23.5	3.5	4,200	9.7	4
1.5	23.5	4	4,100	9.7	3
2.5	23	3.5	4,200	9.8	1
3.5	19	5	5,500	13.1	0
2.5	17	3.5	5,300	12.7	12
3	6.5	2.5	5,400	12.9	15
3	20.5	3.5	5,600	13.2	3
2.5	23	3	5,500	13.1	1
2.5	15	2.5	5,500	13.0	15
2	10	1	5,600	13.1	22
2.5	20	2.5	5,500	13	5
2	23.5	3	5,400	12.9	1
3.5	23	3.5	5,400	12.9	3

Case No.	Day of Experiment	W.B.C. per c.mm.	Non-Lob. Neutro-phil	Lob. Neutro-phil	Baso-phil
D56	68	18,600	2	76.5	0
	72	24,300	2.5	82	0
	80	12,300	0.5	72.5	0
	87	11,000	0.5	71	0
	94	16,700	1.5	75.5	0.5
	101	23,600	2	81.5	0
	106	11,900	0.5	72.5	0
	110	14,300	0.5	75.5	0
	114	17,000	1	78	0
	118	22,300	1.5	81	0
	120	25,100	2.5	84	0
	124	23,200	2	82.5	0
	126	21,700	1	80.5	0.5
	129	17,600	0.5	79	0
	132	16,100	0.5	78.5	0
	135	15,300	0.5	76.5	0

Eosino-phil	Lympho-cyte	Mono-cyte	R.B.C. 10^3 per c.mm.	Haemoglobin g/100ml	E.S.R.
2.5	16.5	2.5	5,300	12.7	14
2	11.5	2	5,400	12.8	23
2	22.5	2.5	5,200	12.5	2
1.5	25	2	5,200	12.4	2
2.5	18	2	5,000	12	11
3	12	1.5	4,900	11.8	26
2.5	22	2.5	4,900	11.8	12
1.5	20.5	2	4,800	11.6	2
2	17	2	4,600	11.2	3
2	14	1.5	4,700	11.3	17
1.5	9.5	2.5	4,600	11	24
2	11.5	2	4,500	10.7	21
2.5	13	2.5	4,300	10.3	18
2	15.5	3	4,200	10.1	15
1.5	16	3.5	4,100	9.9	14
2.5	17.5	3	4,100	9.8	11

Appendix 12

Vaginal Cytology of Experimental Animals.

Case No.	Day of Experiment	Cornified	Epithelial Cells: Eosinophil	Epithelial Cells: Large Basophil	Epithelial Cells: Small Basophil	Blood Cells: Neutrophil	Blood Cells: R.B.C.
D 1	1	0	74	0	26	2	0
	2	0	57	6	37	1	0
	3	2	32	29	37	16	23
	4	23	34	27	16	23	17
	5	53	43	4	0	21	39
	6	59	40	1	0	29	57
	7	68	32	0	0	26	43
	8	73	27	0	0	32	26
	9	84	16	0	0	38	31
	10	87	13	0	0	31	22
D 2	1	0	69	2	31	0	0
	2	0	52	9	39	0	0
	3	0	36	22	42	2	11
	4	2	38	27	33	13	27
	5	29	36	19	16	11	39
	6	44	31	16	9	27	56
	7	69	31	0	0	26	24
	8	73	27	0	0	31	43
	9	86	14	0	0	39	41
	10	89	11	0	0	42	27

Case No.	Day of Experiment	Cornified	Epithelial Cells: Eosinophil	Epithelial Cells: Large Basophil	Epithelial Cells: Small Basophil	Blood Cells: Neutrophil	Blood Cells: R.B.C.
D 3	1	0	75	0	25	0	0
	3	3	51	28	18	2	0
	5	53	39	7	1	0	0
	7	67	32	1	0	19	7
	9	81	19	0	0	9	0
	11	89	11	0	0	8	0
	13	97	3	0	0	2	0
	15	100	0	0	0	0	0
	17	98	2	0	0	0	0
	19	99	1	0	0	2	0
	21	98	2	0	0	7	0
D 4	1	0	73	1	26	0	0
	3	3	48	22	27	0	0
	5	49	21	15	15	9	7
	7	79	19	2	0	17	78
	9	85	15	0	0	37	86
	11	97	3	0	0	23	120
	13	99	1	0	0	31	16
	15	98	2	0	0	24	3
	17	99	1	0	0	9	0

Case No.	Day of Experiment	Cornified	Epithelial Cells			Blood Cells	
			Eosinophil	Large Basophil	Small Basophil	Neutrophil	R.B.C.
D 4	19	100	0	0	0	3	1
	21	98	2	0	0	2	0
	23	99	1	0	0	7	0
	25	99	1	0	0	8	0
	26	99	1	0	0	1	0
D 5	1	0	66	7	27	2	0
	3	11	49	19	21	0	0
	5	48	34	12	6	2	0
	7	76	21	3	0	1	0
	9	87	13	0	0	3	0
	11	94	6	0	0	19	17
	13	98	2	0	0	22	29
	15	99	1	0	0	24	47
	17	98	2	0	0	18	23
	19	99	1	0	0	31	64
	21	98	2	0	0	53	21
	23	100	0	0	0	21	16
	25	99	1	0	0	37	12
	27	100	0	0	0	19	9

			Epithelial Cells			Blood Cells	
Case No.	Day of Experiment	Cornified	Eosinophil	Large Basophil	Small Basophil	Neutrophil	R.B.C.
D 7	1	0	69	0	31	2	0
	3	5	39	22	34	3	0
	5	57	36	5	2	9	0
	7	69	25	6	0	8	2
	9	86	14	0	0	26	19
	10	89	11	0	0	34	53
D 9	1	0	74	0	26	0	0
	5	51	20	15	6	12	17
	9	86	14	0	0	19	28
	13	97	3	0	0	35	64
	17	99	1	0	0	23	37
	21	100	0	0	0	0	0
	25	99	1	0	0	0	0
	30	99	1	0	0	0	0
D 13	1	0	79	0	21	3	0
	3	8	61	3	28	2	0
	5	60	35	1	4	29	112
	7	96	4	0	0	36	193
	9	98	2	0	0	21	126

		EPITHELIAL CELLS				BLOOD CELLS	
Case No.	Day of Experiment	Cornified	Eosinophil	Large Basophil	Small Basophil	Neutrophil	R.B.C.
D 13	11	100	0	0	0	17	54
	13	100	0	0	0	9	17
D 14	1	0	73	0	27	2	0
	2	0	72	0	28	0	0
	3	0	70	0	30	0	0
	4	0	58	0	42	0	0
	5	0	49	0	51	0	0
	6	0	46	0	54	0	0
	7	0	43	0	57	0	0
	8	0	42	2	56	0	0
	9	0	40	7	53	0	0
	10	0	38	12	50	0	0
D 18	1	0	76	0	24	3	0
	3	3	43	29	25	0	0
	5	54	32	12	2	7	39
	7	67	30	3	0	13	56
	9	86	14	0	0	34	41
	11	89	11	0	0	46	67
	12	93	7	0	0	41	19

		EPITHELIAL CELLS				BLOOD CELLS	
Case No.	Day of Experiment	Cornified	Eosinophil	Large Basophil	Small Basophil	Neutrophil	R.B.C.
D 18	13	95	5	0	0	124	5
	14	93	7	0	0	93	0
	15	74	24	0	2	72	0
	16	61	32	0	7	37	0
	17	52	39	0	9	21	0
	18	33	47	0	20	18	0
	19	25	42	0	33	25	0
	20	19	49	1	31	12	0
D 23	1	0	62	2	36	1	0
	3	6	35	28	31	3	0
	5	47	28	11	3	19	13
	7	61	37	2	0	28	9
	9	82	18	0	0	23	35
	11	89	11	0	0	31	43
	13	93	7	0	0	98	2
	15	90	10	0	0	43	0
	17	74	26	0	0	31	0
	19	36	48	0	16	22	0
	21	11	43	0	46	6	0
	23	0	47	0	53	0	0
	25	0	44	6	50	0	0

Case No.	Day of Experiment	EPITHELIAL CELLS				BLOOD CELLS	
		Cornified	Eosinophil	Large Basophil	Small Basophil	Neutrophil	R.E.C.
D 23	27	0	46	10	44	0	0
	30	0	41	16	43	0	0
D 28	1	0	61	0	39	0	0
	5	51	36	10	3	16	10
	9	87	13	0	0	21	56
	13	94	6	0	0	67	11
	17	36	49	0	15	26	0
	21	0	56	0	44	0	0
	25	0	53	2	45	0	0
	29	0	51	3	46	0	0
	33	0	49	7	44	0	0
	37	0	40	12	48	0	0
	41	0	38	9	53	0	0
	45	0	31	13	56	0	0
	49	0	28	14	58	0	0
D 30	1	0	68	0	32	0	0
	5	47	42	6	5	19	2
	9	73	27	0	0	47	11
	13	89	11	0	0	104	0

Case No.	Day of Experiment	EPITHELIAL CELLS				BLOOD CELLS	
		Cornified	Eosinophil	Large Basophil	Small Basophil	Neutrophil	M.B.C.
D 30	17	38	57	0	5	36	0
	21	9	63	0	28	0	0
	25	0	51	0	49	0	0
	29	0	47	0	53	0	0
	33	0	41	0	59	0	0
	37	0	44	0	56	0	0
	41	0	39	0	61	0	0
	45	0	38	3	59	0	0
	49	0	34	10	56	0	0
	53	0	37	13	50	0	0
	57	0	37	22	41	9	0
	61	0	38	17	45	7	0
	65	0	41	20	39	6	0
	69	0	39	16	45	0	0
D 32	1	0	73	0	27	3	0
	5	51	43	5	1	21	37
	9	84	16	0	0	38	31
	13	91	9	0	0	95	0
	17	52	39	0	9	23	0
	21	11	43	0	46	0	0

		EPITHELIAL CELLS				BLOOD CELLS	
Case No.	Day of Experiment	Cornified	Eosinophil	Large Basophil	Small basophil	Neutrophil	M.D.C.
D 32	25	2	47	0	51	0	0
	29	0	46	0	54	0	0
	33	7	42	26	25	0	0
	37	76	17	7	0	31	17
	41	92	8	0	0	46	5
	45	76	24	0	0	13	0
	49	27	46	0	27	0	0
	53	6	47	0	47	0	0
	57	0	49	0	51	0	0
	60	0	53	0	47	0	0
D 34	1	0	67	0	33	0	0
	5	48	35	14	3	17	22
	9	86	14	0	0	9	3
	13	90	10	0	0	75	7
	17	43	46	0	11	39	0
	21	7	54	0	39	2	0
	25	0	47	0	53	0	0
	29	0	42	0	58	0	0
	33	6	40	21	33	0	2
	37	78	18	2	2	20	29
	41	91	9	0	0	9	7

		EPITHELIAL CELLS				BLOOD CELLS	
Case No.	Day of Experiment	Cornified	Eosinophil	Large Basophil	Small Basophil	Neutrophil	R.B.C.
D 34	45	62	35	0	3	17	0
	49	17	51	0	32	0	0
	53	1	53	0	46	0	0
	57	0	47	0	53	0	0
	61	0	45	0	55	0	0
	65	53	27	7	13	7	5
	69	89	11	0	0	53	27
	73	96	4	0	0	113	10
	77	58	31	0	11	0	0
	81	27	43	0	30	0	0
	85	3	49	0	48	0	0
	89	0	47	0	53	0	0
	93	9	41	19	31	0	0
	97	83	16	1	0	29	11
	101	97	3	0	0	36	7
	105	63	31	0	6	2	0
	109	21	48	0	31	0	0
	113	0	56	0	44	0	0
	117	0	51	0	49	0	0
	120	0	46	0	54	0	0

Case No.	Day of Experiment	EPITHELIAL CELLS				BLOOD CELLS	
		Cornified	Eosinophil	Large Basophil	Small Basophil	Neutrophil	R.B.C.
D 34	45	62	35	0	3	17	0
	49	17	51	0	32	0	0
	53	1	53	0	46	0	0
	57	0	47	0	53	0	0
	61	0	45	0	55	0	0
	65	53	27	7	13	7	5
	69	89	11	0	0	53	27
	73	96	4	0	0	113	10
	77	58	31	0	11	0	0
	81	27	43	0	30	0	0
	85	3	49	0	48	0	0
	89	0	47	0	53	0	0
	93	9	41	19	31	0	0
	97	83	16	1	0	29	11
	101	97	3	0	0	36	7
	105	63	31	0	6	2	0
	109	21	48	0	31	0	0
	113	0	56	0	44	0	0
	117	0	51	0	49	0	0
	120	0	46	0	54	0	0

Case No.	Day of Experiment	EPITHELIAL CELLS				BLOOD CELLS	
		Cornified	Eosinophil	Large Basophil	Small Basophil	Neutrophil	R.B.C.
D 36	1	0	72	0	28	0	0
	5	57	35	6	2	9	0
	9	89	11	0	0	23	7
	13	96	4	0	0	19	0
	17	59	28	0	13	2	0
	21	13	57	0	30	0	0
	25	1	58	0	41	0	0
	29	0	47	0	53	0	0
	33	11	41	29	19	0	0
	37	71	26	3	0	73	29
	41	93	7	0	0	56	43
	45	79	19	0	2	10	0
	49	21	43	0	36	0	0
	53	0	56	0	44	0	0
	57	1	51	0	48	0	0
	61	0	46	0	54	0	0
	65	56	31	9	4	20	2
	69	91	9	0	0	72	186
	73	97	3	0	0	191	74
	77	61	27	0	12	7	0
	81	31	50	0	19	0	0
	85	6	57	0	37	0	0

		EPITHELIAL CELLS				BLOOD CELLS	
Case No.	Day of Experiment	Cornified	Eosinophil	Large Basophil	Small Basophil	Neutrophil	R.B.C.
D 36	89	0	53	0	47	0	0
	93	11	42	16	31	0	0
	97	86	14	0	0	21	0
	101	98	2	0	0	49	0
	105	67	26	0	7	17	0
	109	25	47	0	28	0	0
	113	0	59	0	41	0	0
	117	1	57	0	42	0	0
	121	0	47	0	53	0	0
	125	52	30	11	7	0	0
	129	94	6	0	0	13	29
	133	98	2	0	0	37	46
	137	64	29	0	7	16	2
	141	37	51	0	12	0	0
	145	0	63	0	37	0	0
	149	0	52	0	48	0	0
	153	14	43	10	33	0	0
	157	89	11	0	0	29	15
	161	99	1	0	0	78	31
	165	68	27	0	5	34	9
	169	22	49	0	29	0	0
	173	3	53	0	44	0	0

Case No.	Day of Experiment	EPITHELIAL CELLS				BLOOD CELLS	
		Cornified	Eosinophil	Large Basophil	Small Basophil	Neutrophil	R.B.C.
D 36	177	0	50	0	50	0	0
	180	0	41	0	59	0	0
D 40	1	0	68	3	29	0	0
	9	88	12	0	0	37	41
	17	61	27	0	12	0	0
	25	2	57	0	41	0	0
	33	13	42	21	24	7	13
	41	94	6	0	0	117	49
	49	23	46	0	31	0	0
	57	0	53	0	47	0	0
	65	59	29	10	2	39	19
	73	96	4	0	0	67	93
	81	34	52	0	14	0	0
	89	0	57	0	43	0	0
	97	87	13	0	0	25	74
	105	69	28	0	3	19	0
	113	0	61	0	39	0	0
	121	0	43	0	57	0	0
	129	95	5	0	0	73	192
	137	60	33	0	7	7	18
	145	0	61	0	39	0	0

		EPITHELIAL CELLS				BLOOD CELLS	
Case No.	Day of Experiment	Cornified	Eosinophil	Large Basophil	Small Basophil	Neutrophil	R.B.C.
D 40	153	16	46	13	25	11	3
	157	86	14	0	0	28	19
	161	93	7	0	0	35	48
	165	47	38	0	15	13	18
	169	9	47	0	44	0	0
	173	0	55	0	45	75	0
	177	0	52	0	48	115	0
	180	0	45	0	55	174	0
D 43	1	0	65	0	35	0	0
	3	7	37	24	32	17	0
	5	49	26	9	16	36	11
	7	67	31	2	0	49	52
	9	83	17	0	0	41	63
	11	94	6	0	0	37	53
	13	89	11	0	0	247	37
	15	71	27	0	2	21	0
	17	33	46	0	21	3	0
	19	7	59	0	34	0	0
	21	2	57	0	41	0	0
	23	0	52	0	48	0	0
	25	0	47	0	53	0	0

		EPITHELIAL CELLS				BLOOD CELLS	
Case No.	Day of Experiment	Cornified	Eosinophil	Large Basophil	Small Basophil	Neutrophil	R.B.C.
D 43	27	0	49	2	49	0	0
	30	0	47	1	52	0	0
D 44	1	0	71	0	29	0	0
	5	51	41	5	3	27	13
	9	86	14	0	0	39	47
	13	93	7	0	0	97	0
	17	51	39	0	10	20	0
	21	9	47	0	44	0	0
	25	0	49	0	51	0	0
	29	0	46	0	54	0	0
	33	11	45	23	21	0	0
	37	77	21	2	0	11	71
	41	95	5	0	0	17	27
	45	71	23	0	6	16	0
	49	29	47	0	24	0	0
	53	3	56	0	41	0	0
	57	0	53	0	47	0	0
	60	0	49	0	51	0	0

Case No.	Day of Experiment	EPITHELIAL CELLS: Cornified	Eosinophil	Large Basophil	Small Basophil	BLOOD CELLS: Neutrophil	R.B.C.
D 46	1	0	75	0	25	0	0
	9	88	12	0	0	27	0
	17	53	33	0	14	0	0
	25	0	59	0	41	0	0
	33	13	42	21	24	9	11
	41	95	5	0	0	70	87
	49	20	45	0	35	0	0
	57	0	56	0	44	0	0
	65	56	32	7	5	23	16
	69	92	8	0	0	75	120
	73	97	3	0	0	123	76
	77	62	29	0	9	0	0
	81	30	53	0	17	0	0
	85	4	55	0	41	0	0
	89	0	53	0	47	26	0
	93	9	44	23	24	329	0
	97	86	14	0	0	19	10
	101	99	1	0	0	127	39
	105	68	24	0	8	9	0
	109	23	47	0	30	0	0
	113	2	58	0	40	29	0
	117	0	53	0	47	76	0
	120	0	49	0	51	63	0

		EPITHELIAL CELLS				BLOOD CELLS	
Case No.	Day of Experiment	Cornified	Eosinophil	Large Basophil	Small Basophil	Neutrophil	R.B.C.
D 51	1	0	72	0	28	0	0
	9	89	11	0	0	23	84
	17	63	31	0	6	0	0
	25	0	57	0	43	0	0
	33	10	47	19	24	20	71
	41	95	5	0	0	91	17
	49	25	47	0	28	0	0
	57	0	53	0	47	0	0
	65	57	33	6	4	21	35
	73	95	5	0	0	69	71
	81	37	54	0	9	0	0
	89	0	57	0	43	0	0
	93	8	46	17	29	17	0
	97	88	12	0	0	43	87
	101	98	2	0	0	71	39
	105	65	27	0	8	7	0
	109	22	49	0	29	0	0
	113	0	56	0	44	32	0
	117	0	53	0	47	47	0
	121	0	51	0	49	75	0
	125	0	47	0	53	>1,000	0

Case No.	Day of Experiment	EPITHELIAL CELLS				BLOOD CELLS	
		Cornified	Eosinophil	Large Basophil	Small Basophil	Neutrophil	R.B.C.
D 51	129	0	45	0	55	>1,000	0
	133	0	41	0	59	271	0
	135	0	43	0	57	23	0

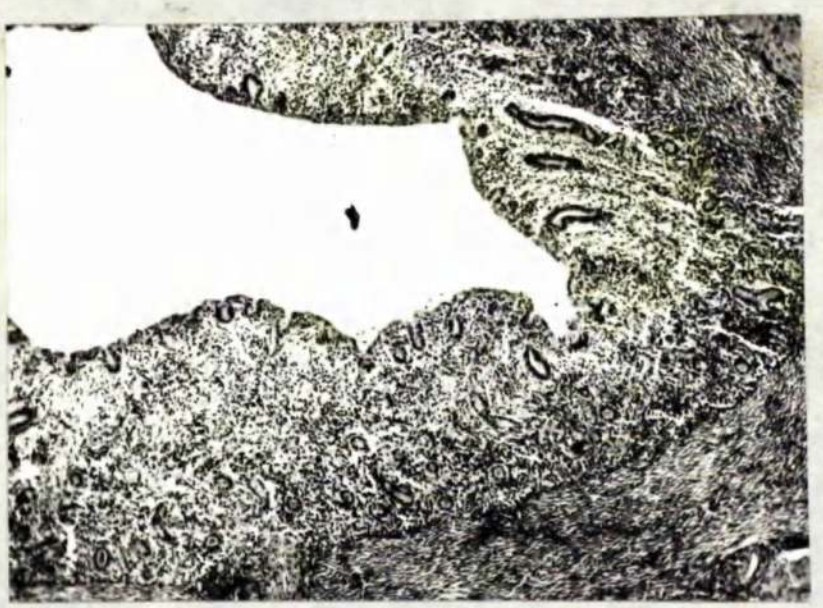

Fig. 1. Endometrium of normal bitch during anoestrus. H. and E. x 35.

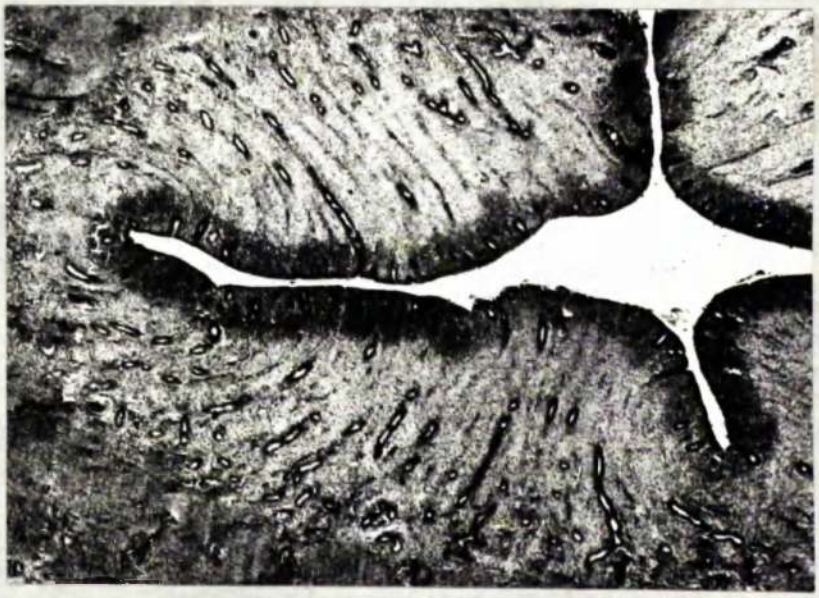

Fig. 2. Endometrium of normal bitch during oestrus. H. and E. x 35.

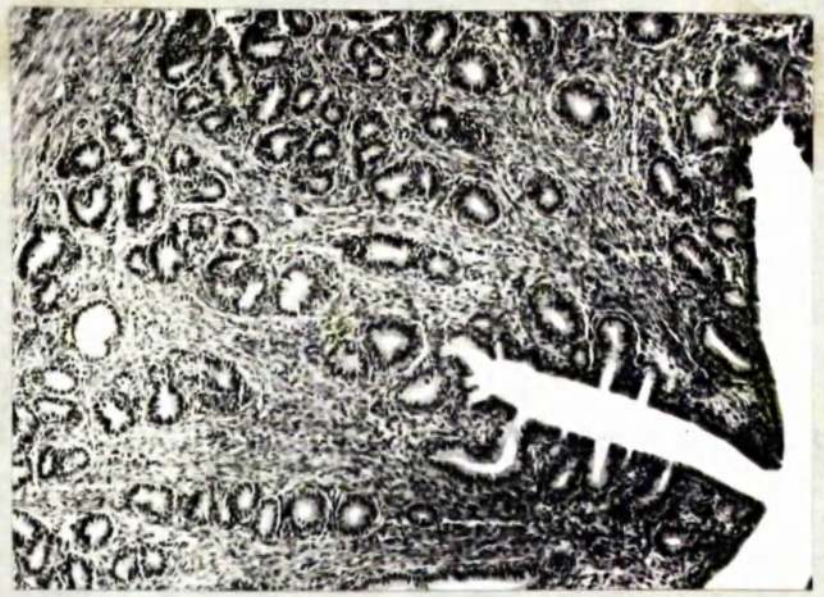

Fig. 3. Endometrium of normal bitch during metoestrus. H. and E. x 110.

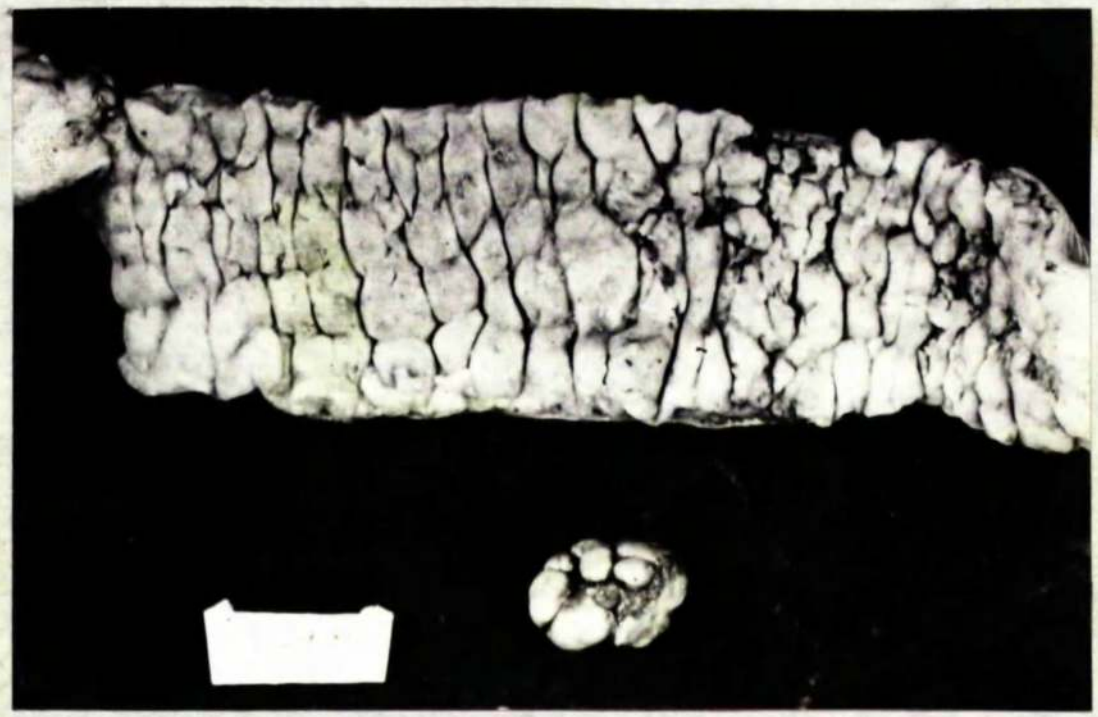

Fig. 6. Canine Uterus. Irregular ridging of endometrium in cystic glandular hyperplasia.

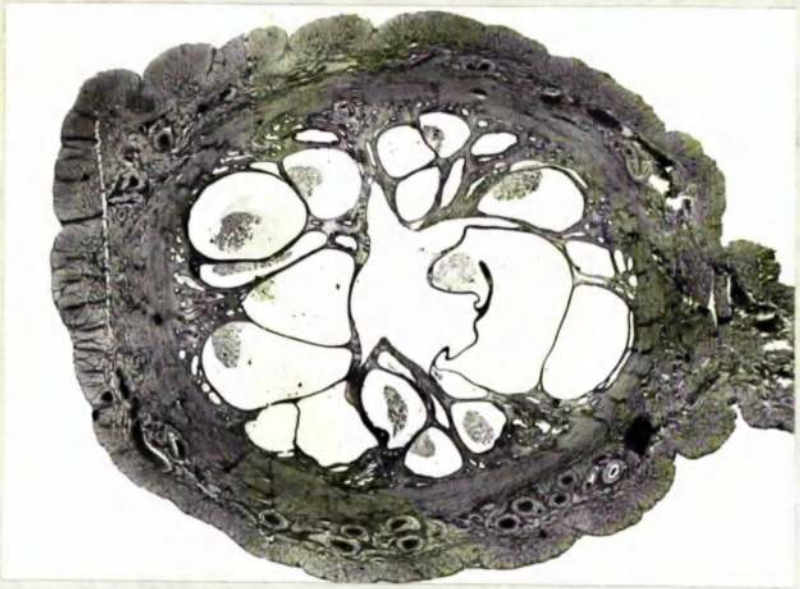

Fig. 7. Cystic Glandular Hyperplasia of the endometrium. H. and E. x 10.

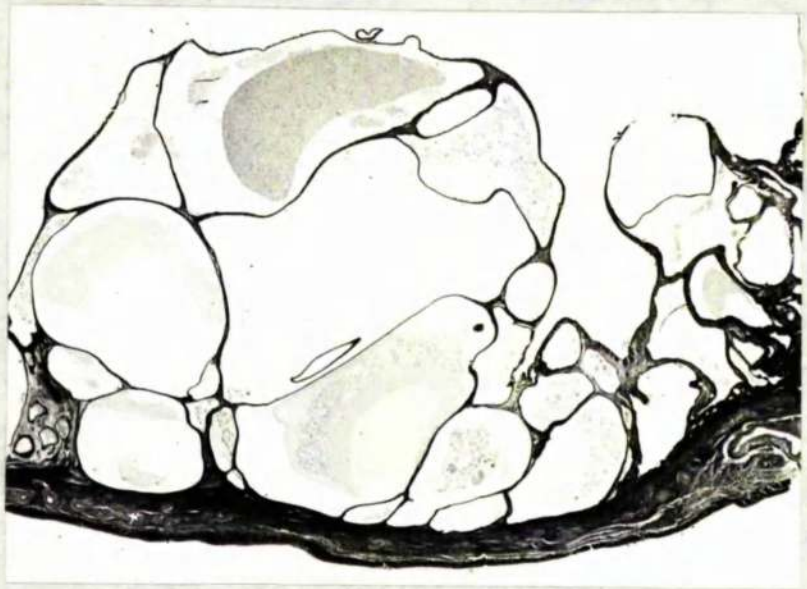

Fig. 8. Cystic dilatation of all glands. H. and E. x 10.

Fig. 9. Cystic glandular hyperplasia; division into two distinct layers of cysts. H. and E. x 35.

Fig. 10. Cystic glandular hyperplasia; papillary projections into cyst lumen. H. and E. x 35.

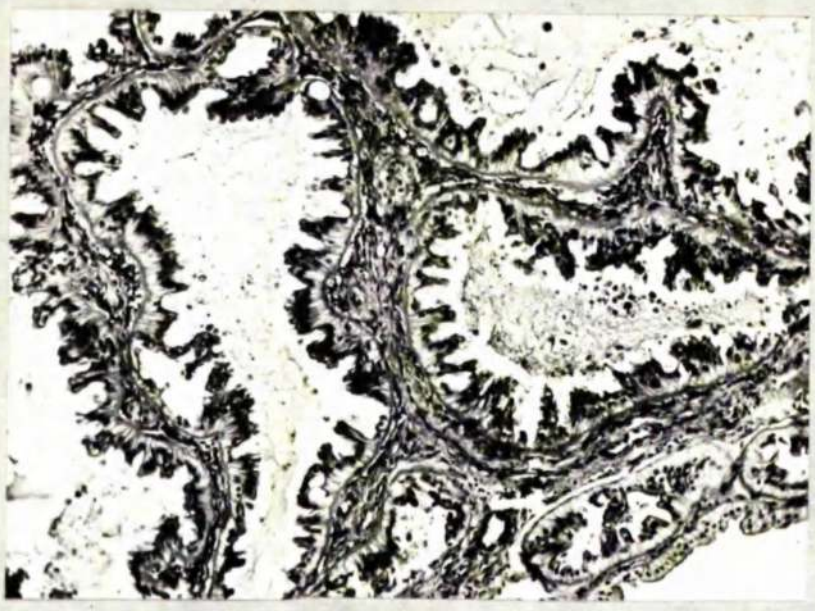

Fig. 11. Cystic glandular hyperplasia; tall, highly secretory epithelium of crypts and surface. H. and E. x 110.

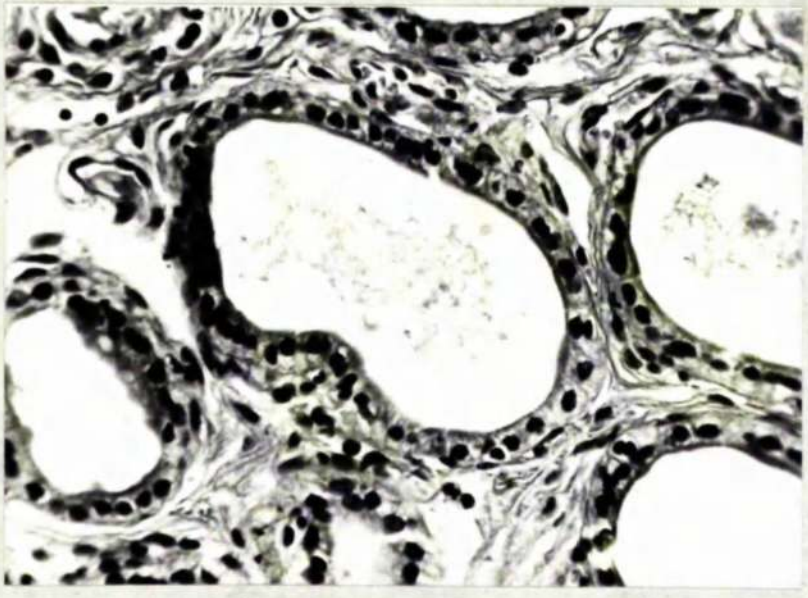

Fig. 12. Cystic glandular hyperplasia; basal cysts lined by cuboidal epithelium. H. and E. x 400.

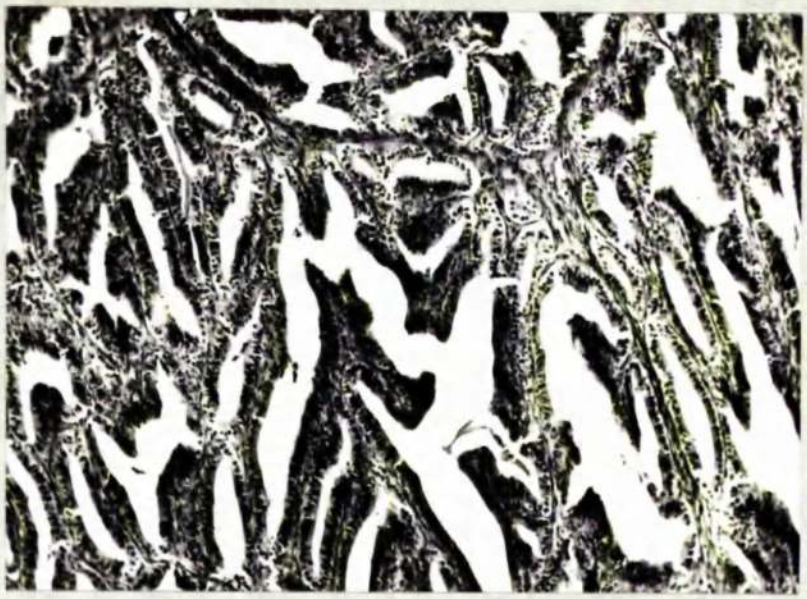

Fig. 13. Cystic glandular hyperplasia; pseudo-adenomatous form. H. and E. x 110.

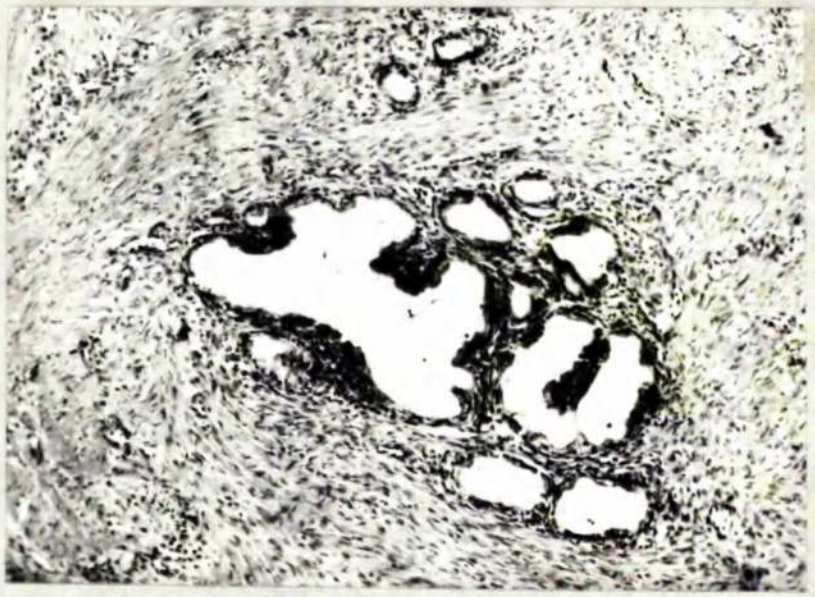

Fig. 14. Focus of adenomyosis in the stratum vasculare. H. and E. x 110.

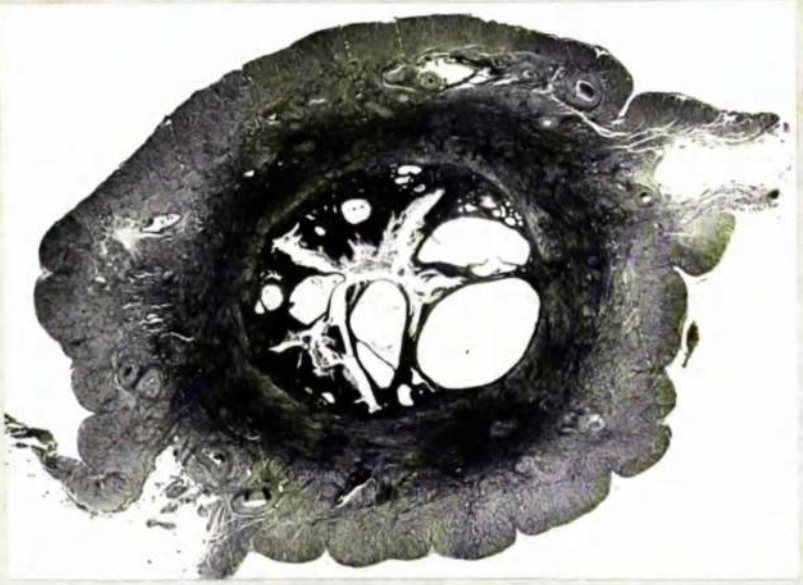

Fig. 17. Cystic glandular hyperplasia of the endometrium with plasma cell infiltration of the stroma. H. and E. x 9.

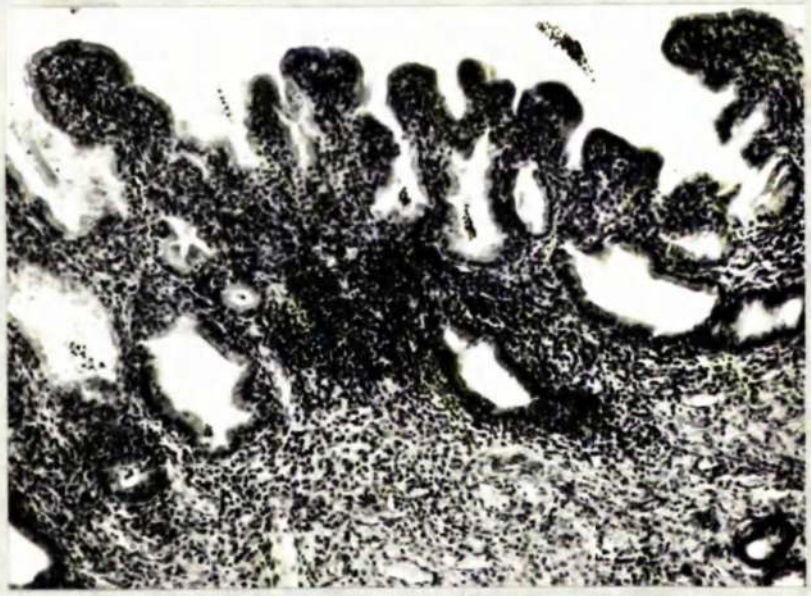

Fig. 18. Formation of superficial papillomata and plasma cell infiltration of the stroma. H. and E. x 110.

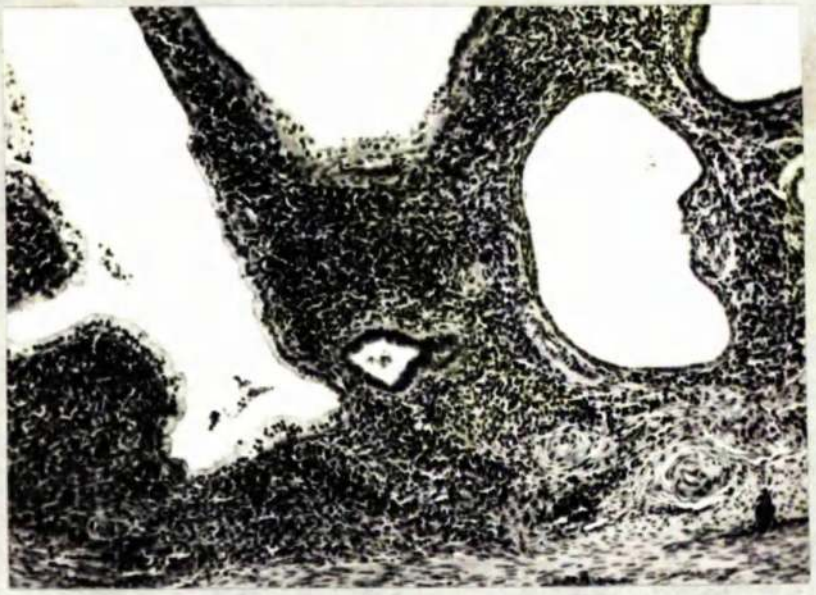

Fig. 19. Plasma cell infiltration round basal cysts; absence of tissue destruction. H. and E. x 110.

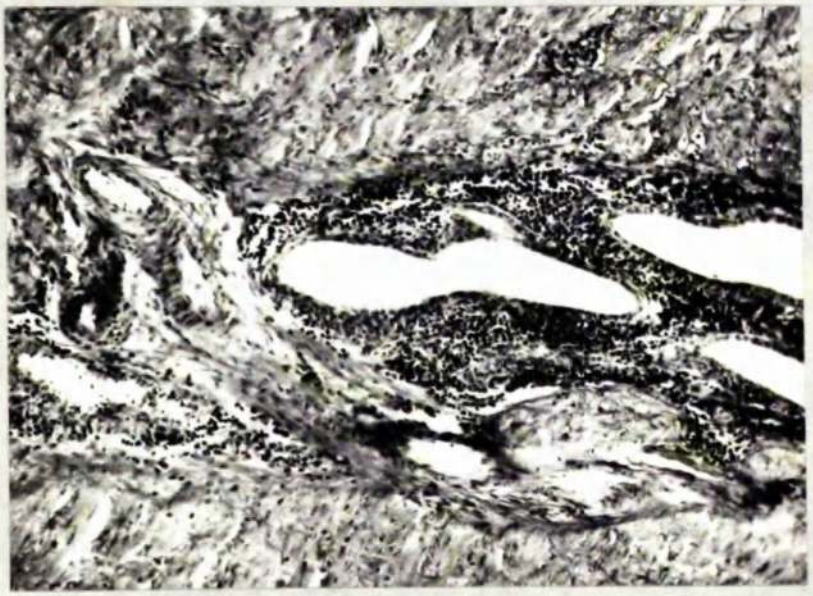

Fig. 20. Plasma cell surrounding focus of adenomyosis in inner myometrium. H. and E. x 110.

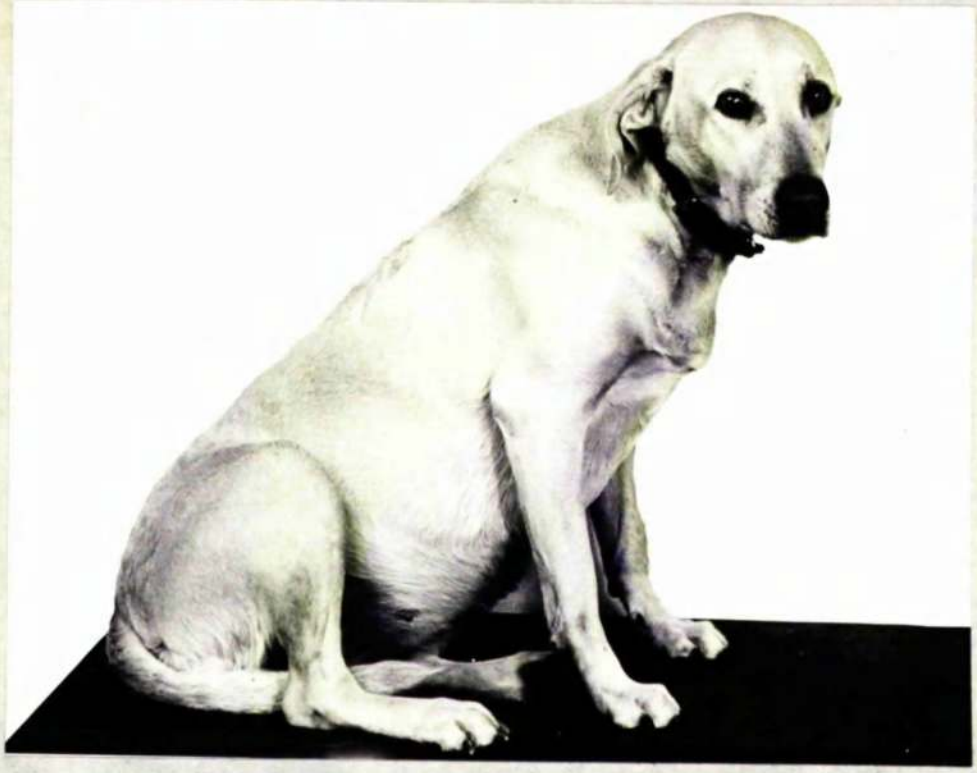

Fig. 23. Case of acute endometritis; abdominal distension.

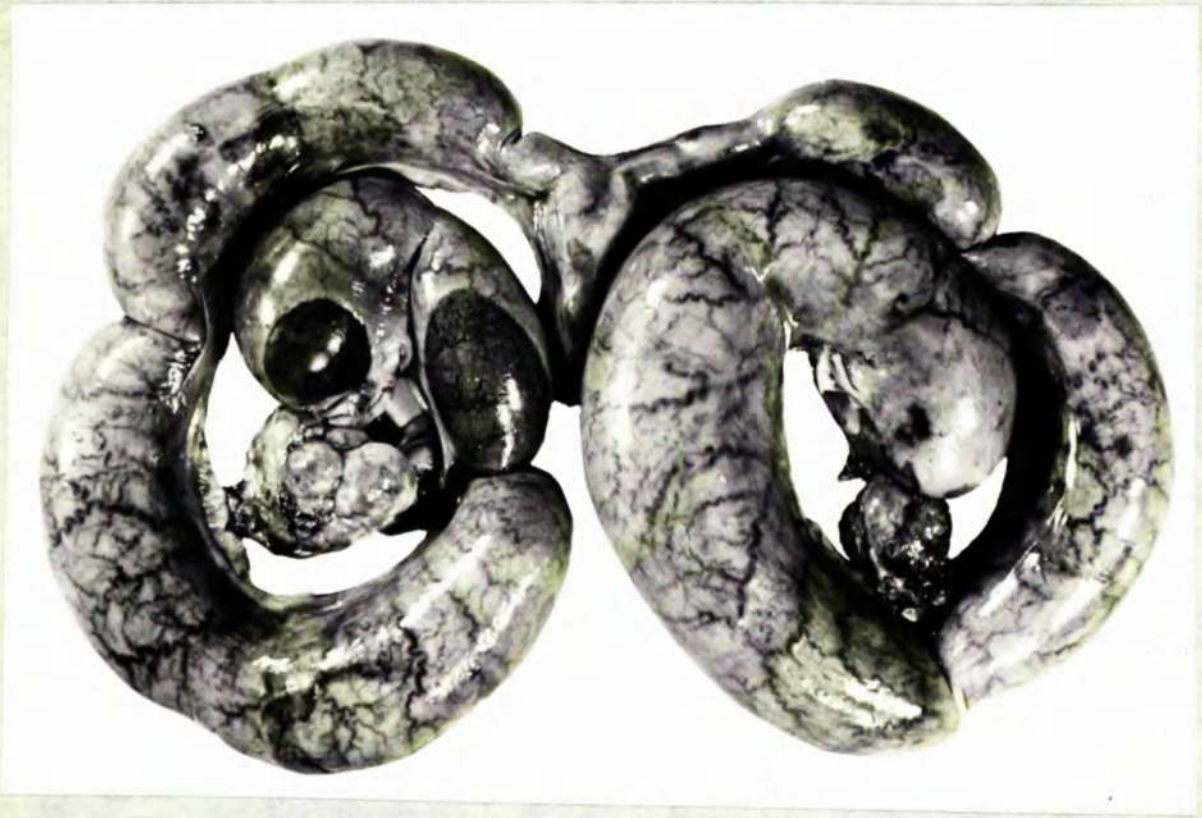

Fig. 24. Acute endometritis; gross distended and coiled uterus.

Fig. 25. Mild acute endometritis; division of cysts into two layers. H. and E. x 30.

Fig. 26. Mild acute endometritis; pseudo-stratification of superficial epithelium; active secretion. H. and E. x 110.

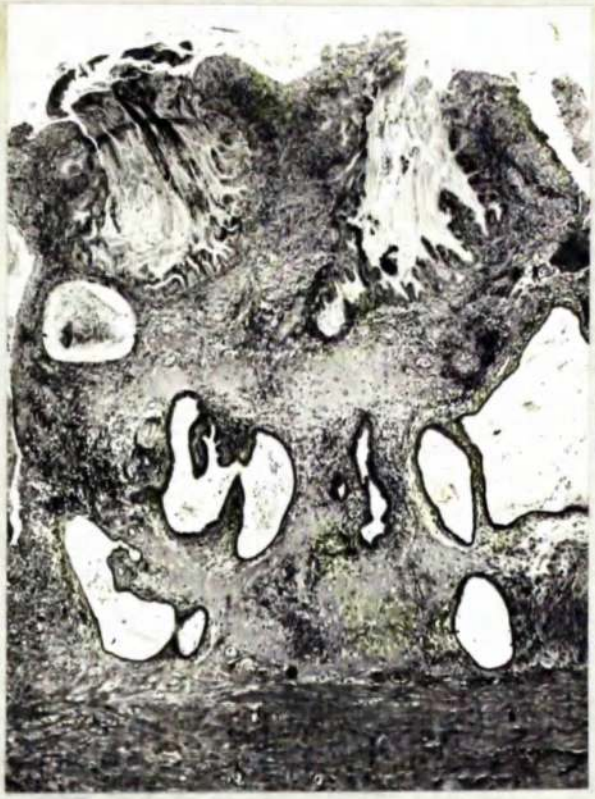

Fig. 27. Acute endometritis; marked oedema of basal zones. H. and E. x 35.

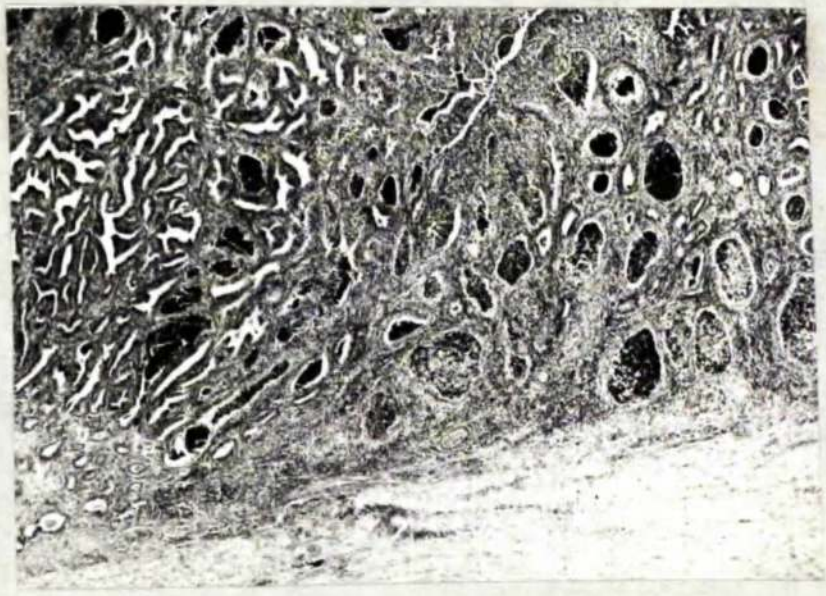

Fig. 28. Severe acute endometritis; blockage of basal cysts with inflammatory exudate; early abscess formation. H. and E. x 35.

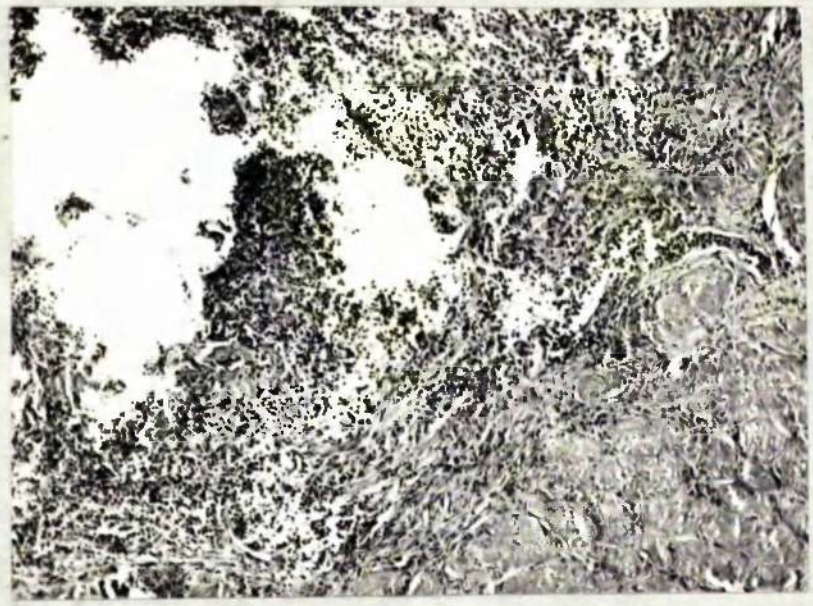

Fig. 29. Rupture of basal abscess with endometrial ulceration. H. and E. x 110.

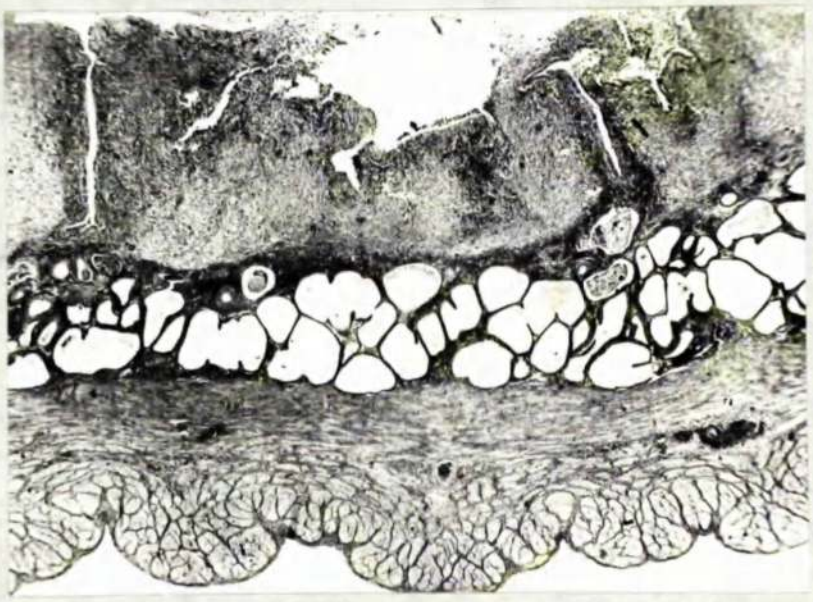

Fig. 30. Recurrent acute endometritis; superficial inflammatory reaction separated from basal cysts by layer of fibrous tissue. H. and E. x 20.

Fig. 31. Severe acute metritis; necrosis of muscle fibres and diffuse neutrophil infiltration. H. and E. x 110.

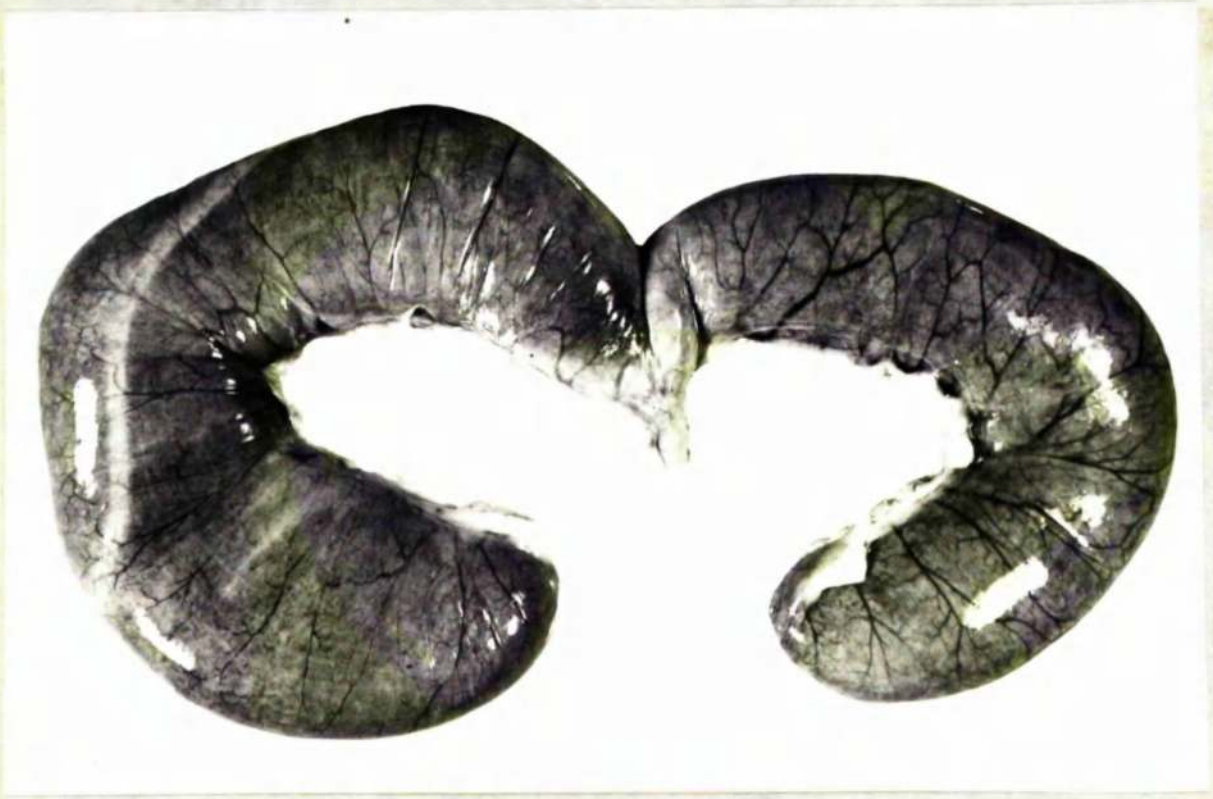

Fig. 34. Chronic endometritis; gross distension; small, tightly constricted cervix.

Fig. 35. Chronic endometritis; atrophy of all layers of the uterine wall; diffuse round cell infiltration of the endometrium. H. and E. x 35.

Fig. 36. Chronic endometritis; squamoid metaplasia of the superficial epithelium. H. and E. x 110.

Fig. 37. Chronic endometritis: squamoid metaplasia in adenomyotic gland. H. and E. x 35.

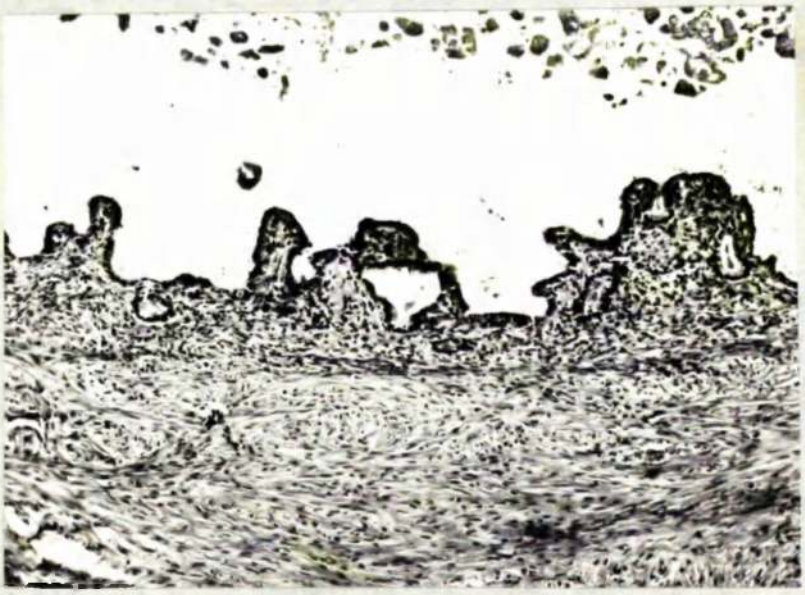

Fig. 38. Chronic endometritis: atrophy of endometrium: poor differentiation of the endometrial - myometrial boundary. H. and E. x 110.

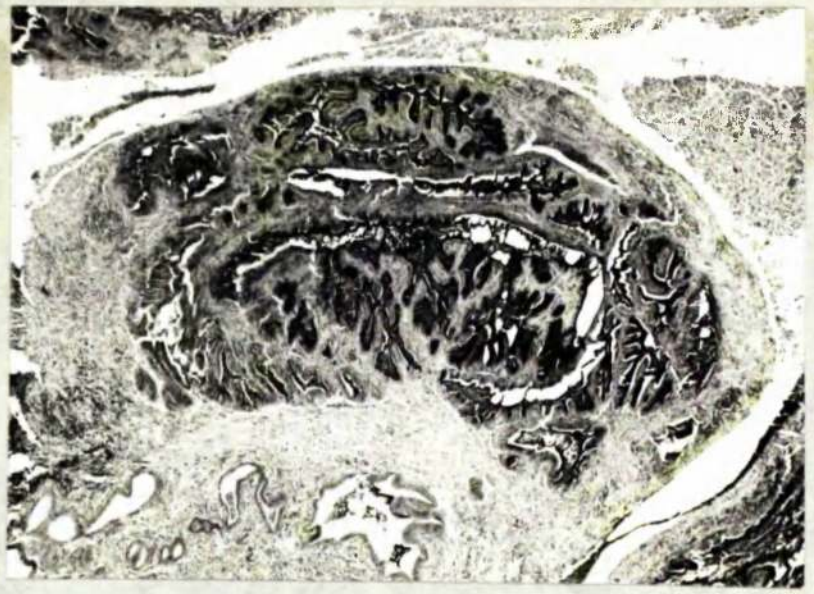

Fig. 39. Acute endometritis: marked secretory activity in superficial cysts. Southgate's mucicarmine x 35.

Fig. 40. Multicystic ovary associated with acute endometritis.

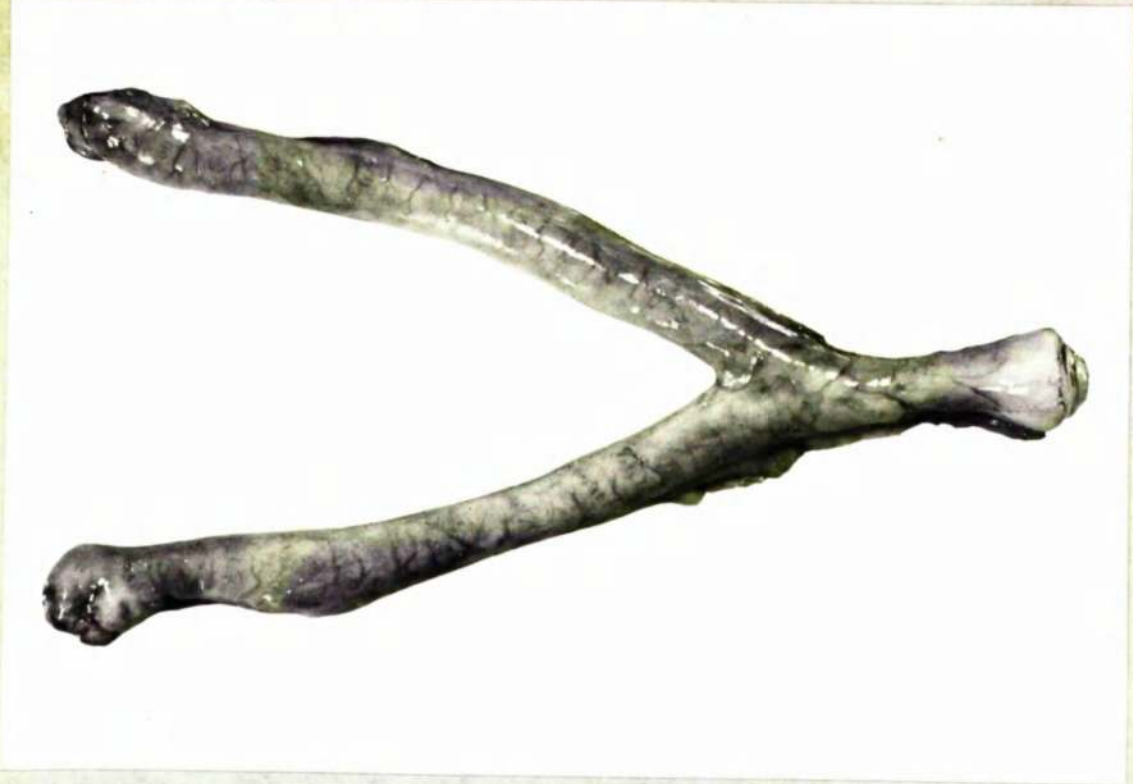

Fig. 41. Case D1. Rounded tense uterus.

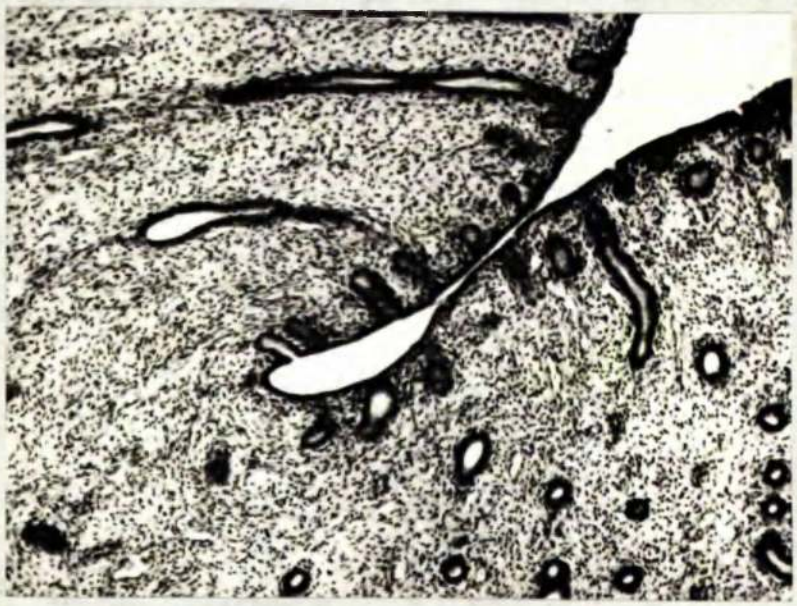

Fig. 42. Case D1: swollen oedematous endometrium. H. and E. x 110.

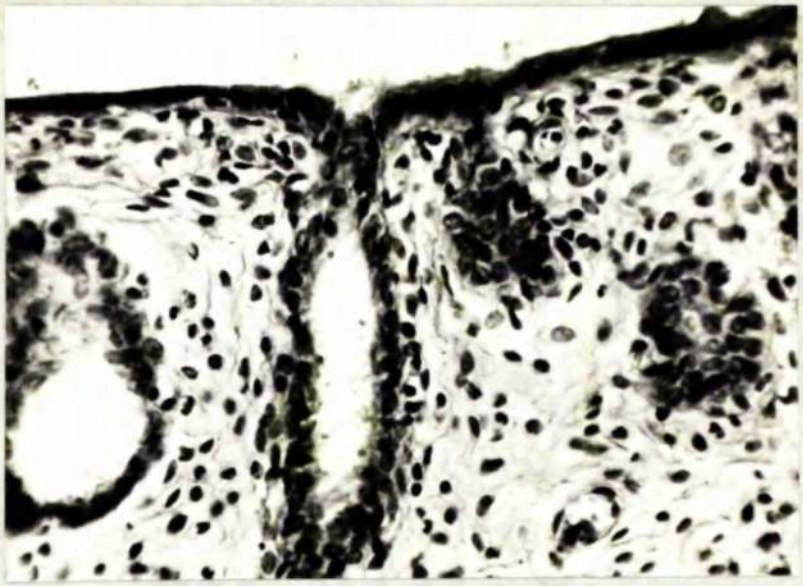

Fig. 43. Case D1: cuboidal superficial epithelium: bottle-shaped crypt. H. and E. x 400.

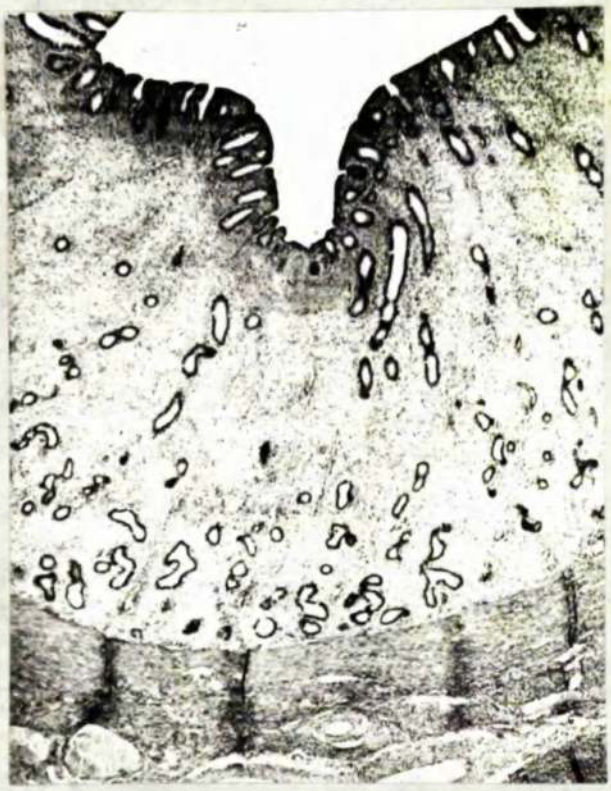

Fig. 44. Case D4: swollen oedematous endometrium: slight dilatation of glands. H. and E. x 35.

Fig. 45. Case D4: increased height and close packing of superficial and crypt epithelium. H. and E. x 400.

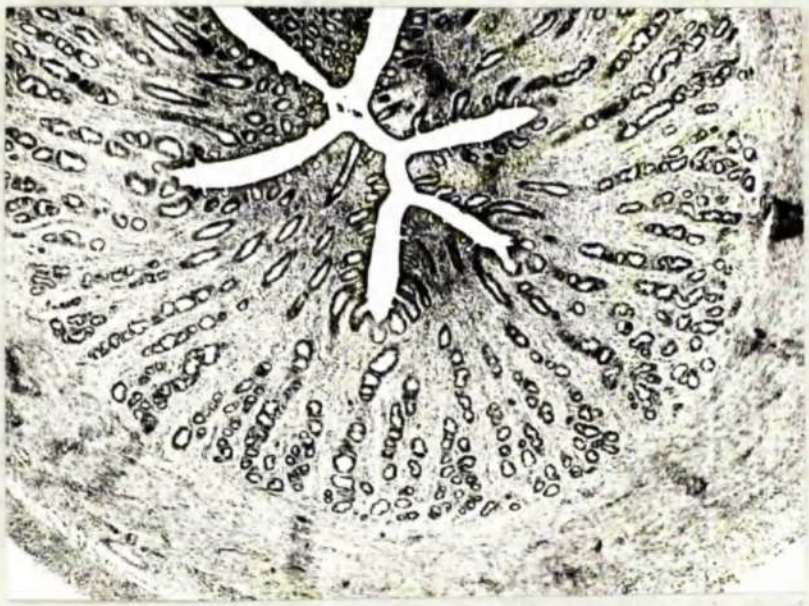

Fig. 46. Case D10: increased number of glands. H. and E. x 35.

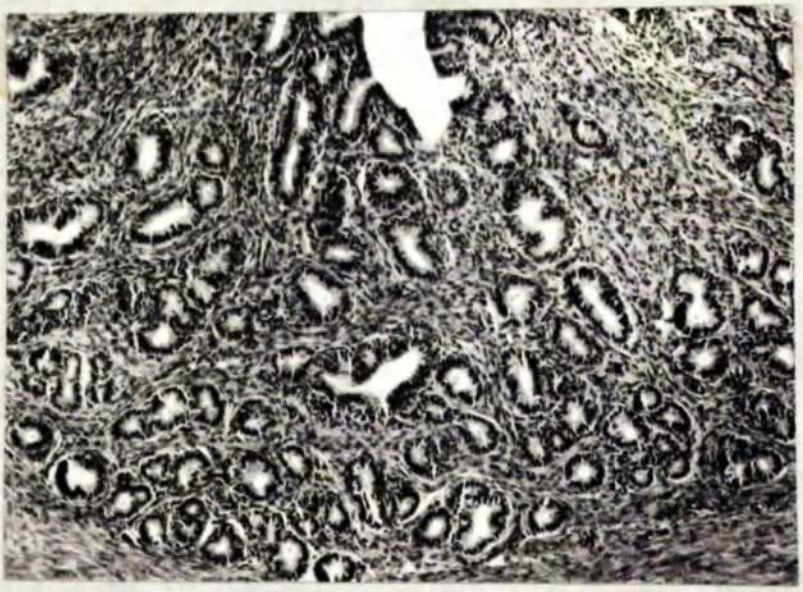

Fig. 47. Case D10: dense stroma: absence of oedema: branching of glands. H. and E. x 110.

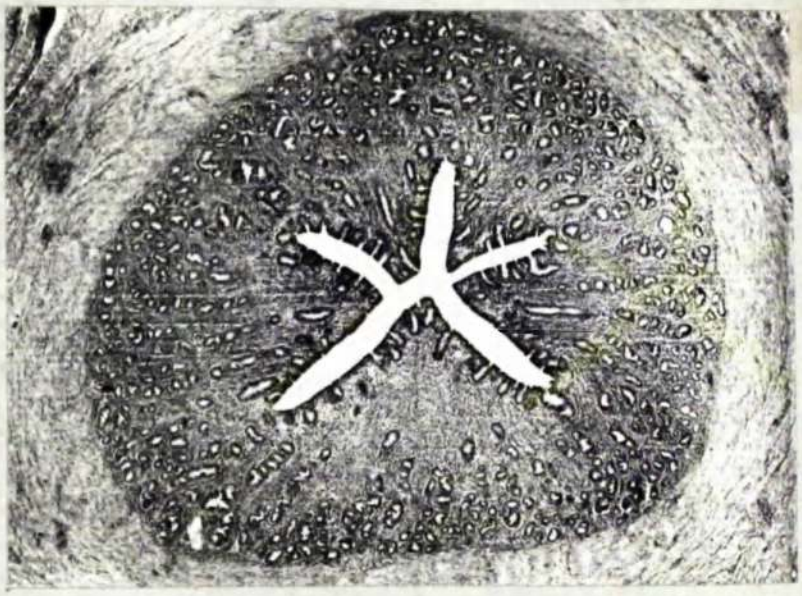

Fig. 48. Case D18: swelling of endometrium: increased number of glands. H. and E. x 30.

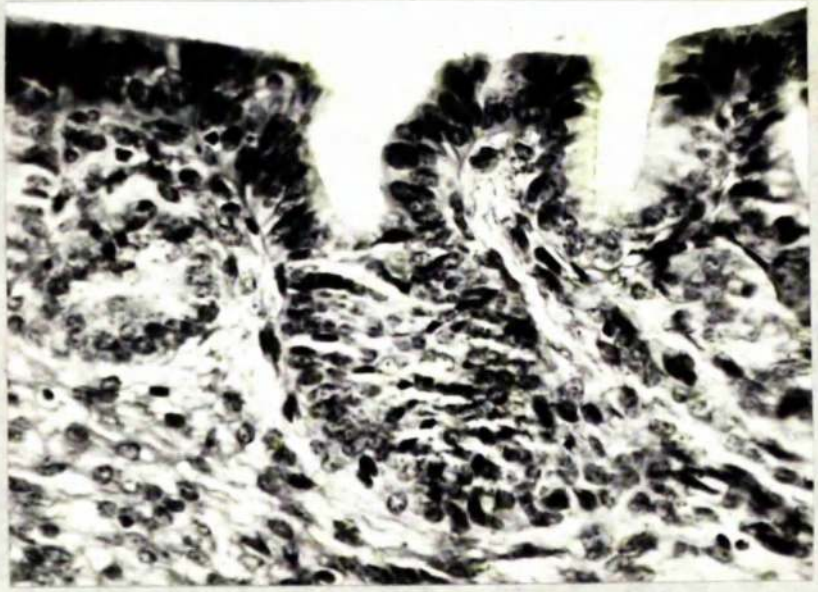

Fig. 49. Case D18: hypertrophy of superficial epithelial cells: both submuclear and supramuclear vacuolation. H. and E. x 400.

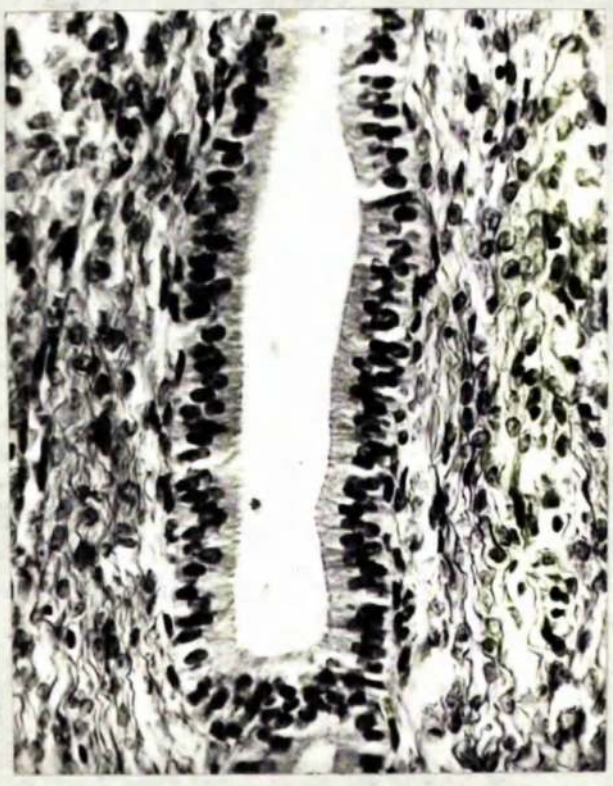

Fig. 50. Case D18: increased size of tubule: tall, columnar epithelium: cytoplasmic vacuolation. H. and E. x 400.

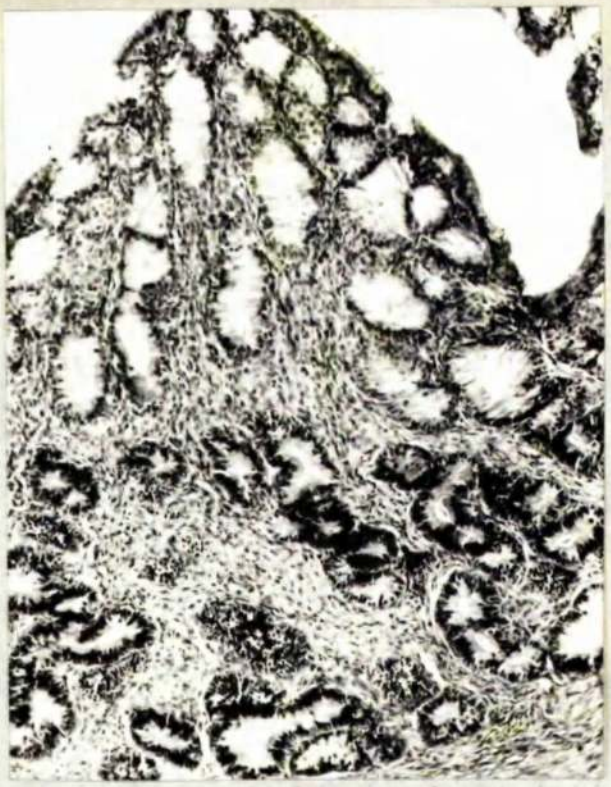

Fig. 51. Case D22: increased width of crypt zone. H. and E. x 110.

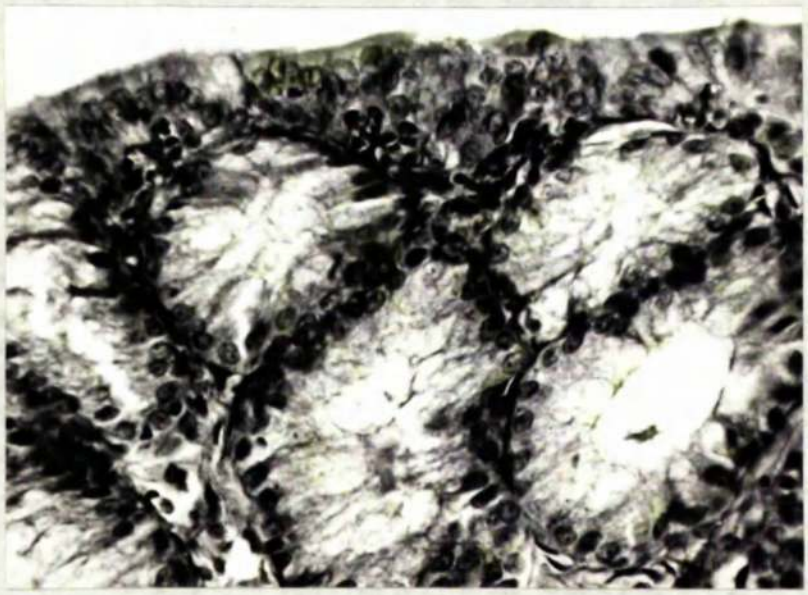

Fig. 52. Case D22: tall pseudo-stratified superficial epithelium: marked vacuolation of crypt cells. H. and E. x 400.

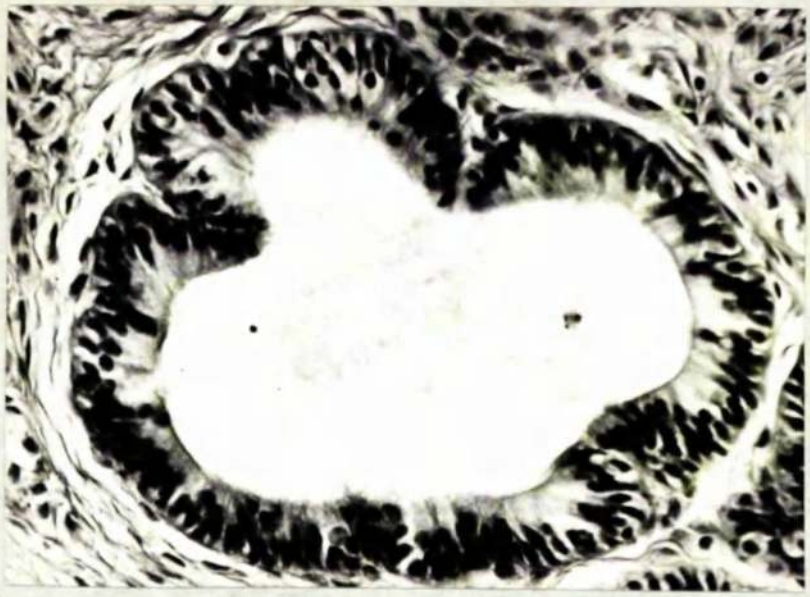

Fig. 53. Case D22: basalis glands: deeply basophilic nuclei: secretion in lumina. H. and E. x 400.

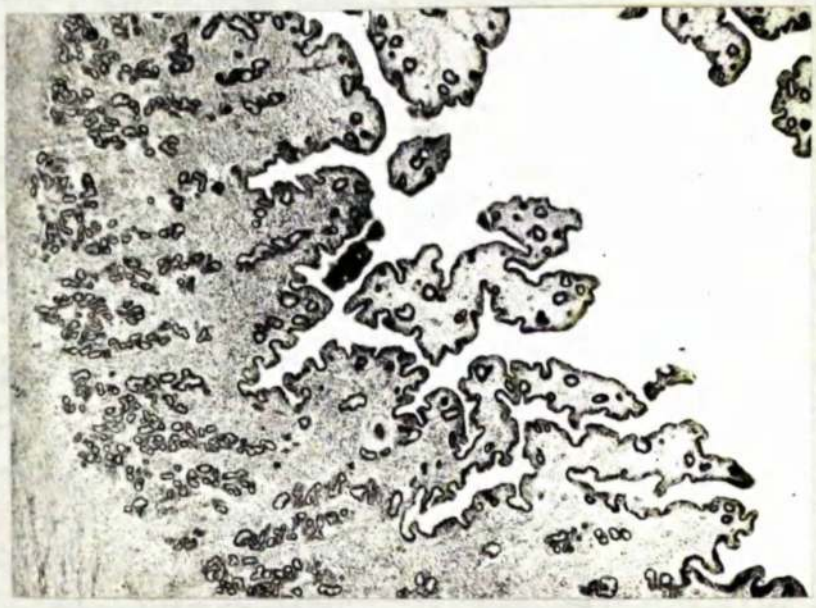

Fig. 54. Case D26: superficial papillomata with few glands. H. and E. x 35.

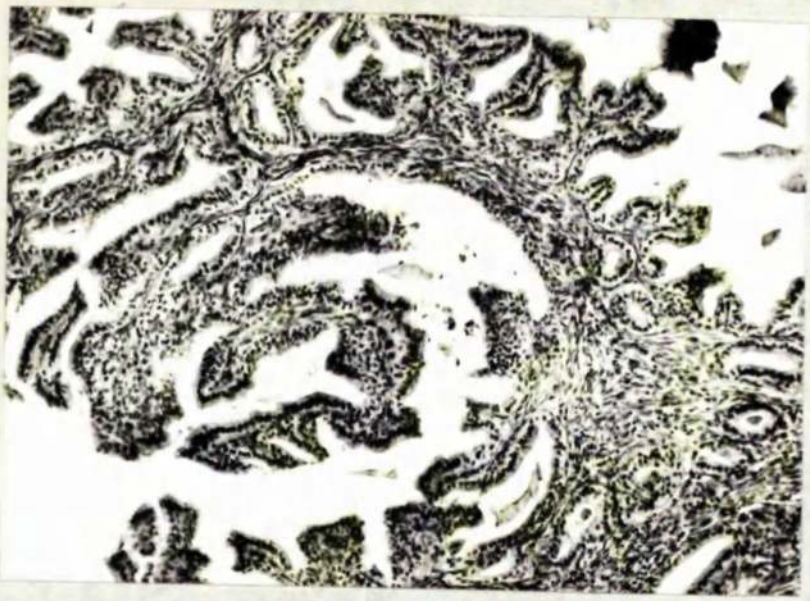

Fig. 55. Case D29: overgrowth of crypt zone: formation of superficial papillomata. H. and E. x 110.

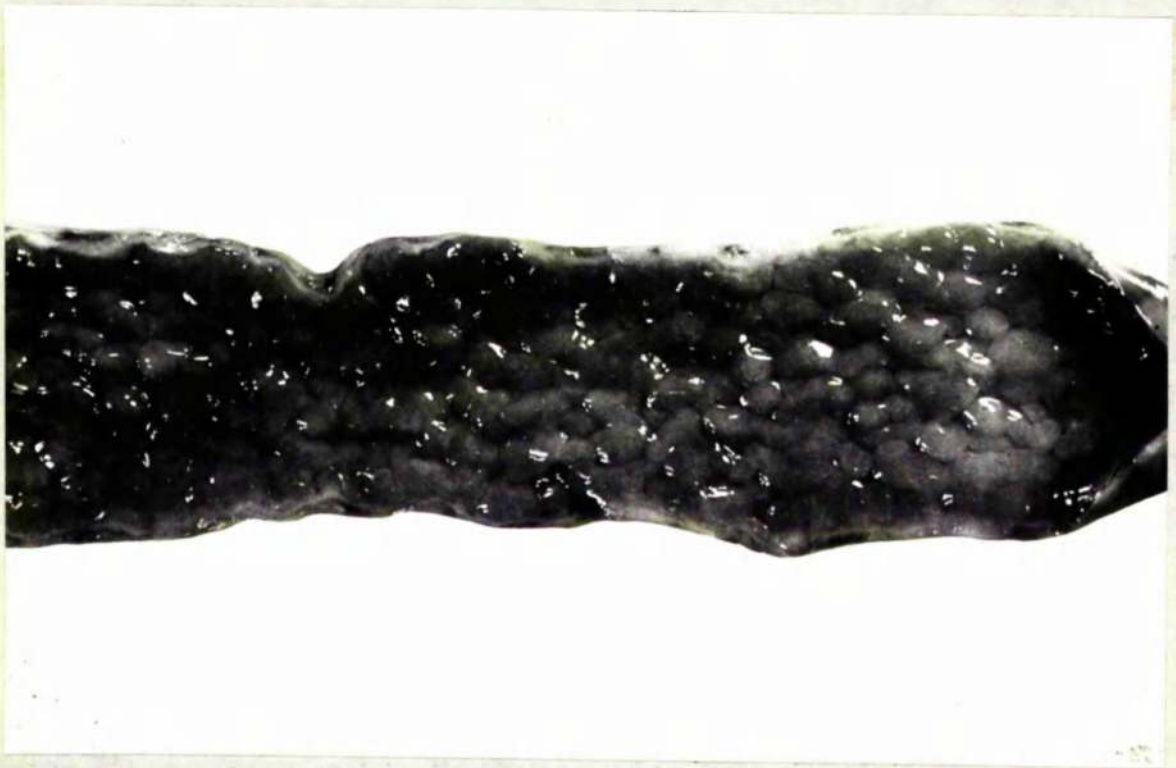

Fig. 56. Case D30: irregular, nodular thickening of the endometrium.

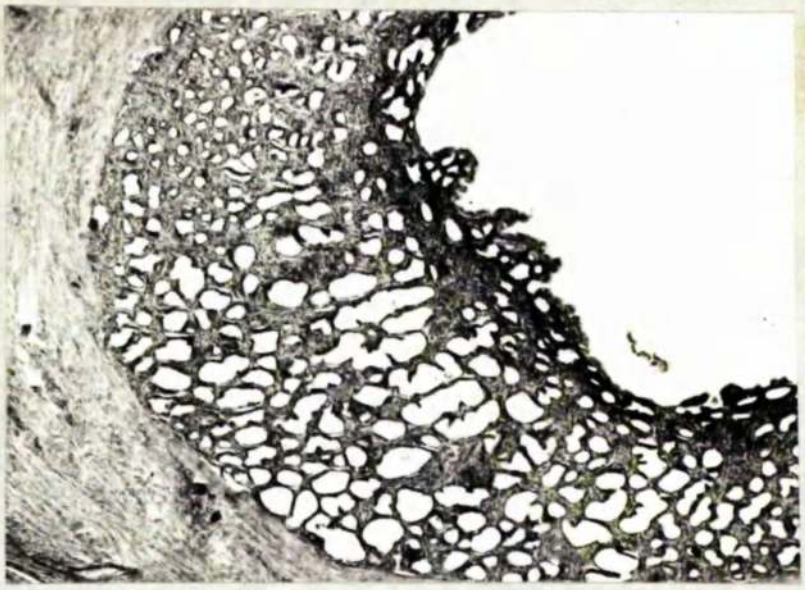

Fig. 57. Case D30: cystic glandular dilatation of all strata of endometrium. H. and E. x 35.

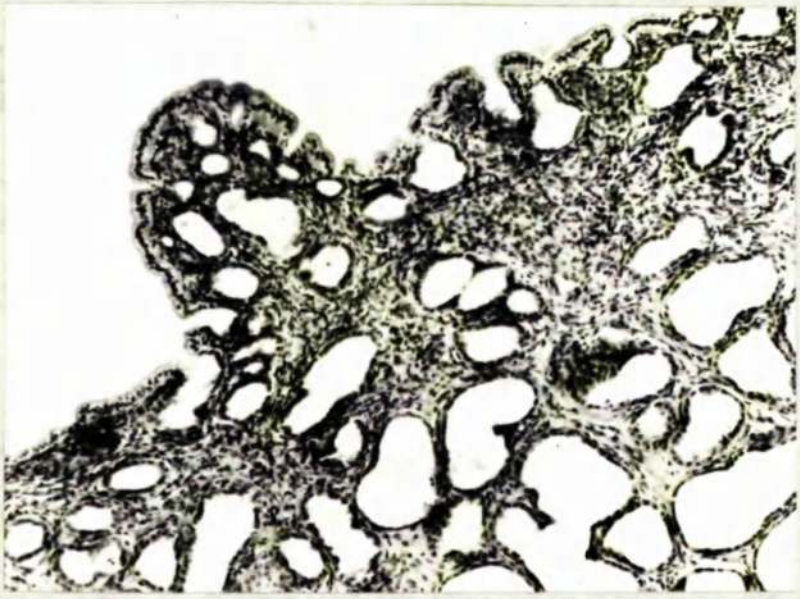

Fig. 58. Case D30: tall, fatty surface epithelium: small superficial papillomata. H. and E. x 110.

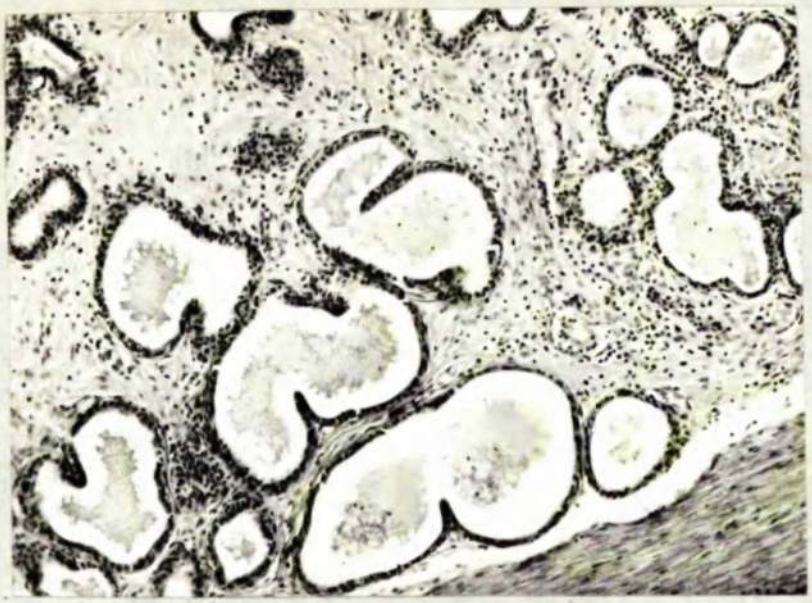

Fig. 59. Case D30: basal cysts: low, cuboidal epithelium: secretion in lumina. H. and E. x 110.

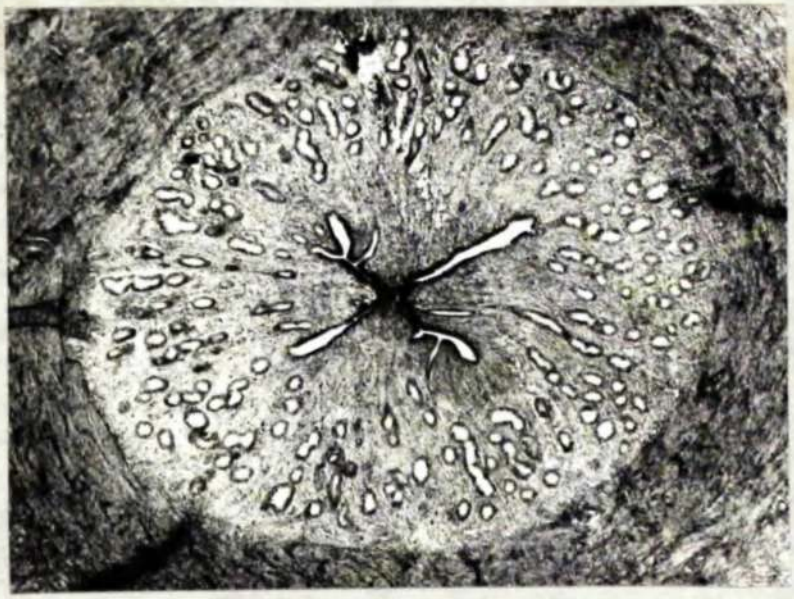

Fig. 60. Case D32: swollen endometrium: branching and dilatation of glands. H. and E. x 35.

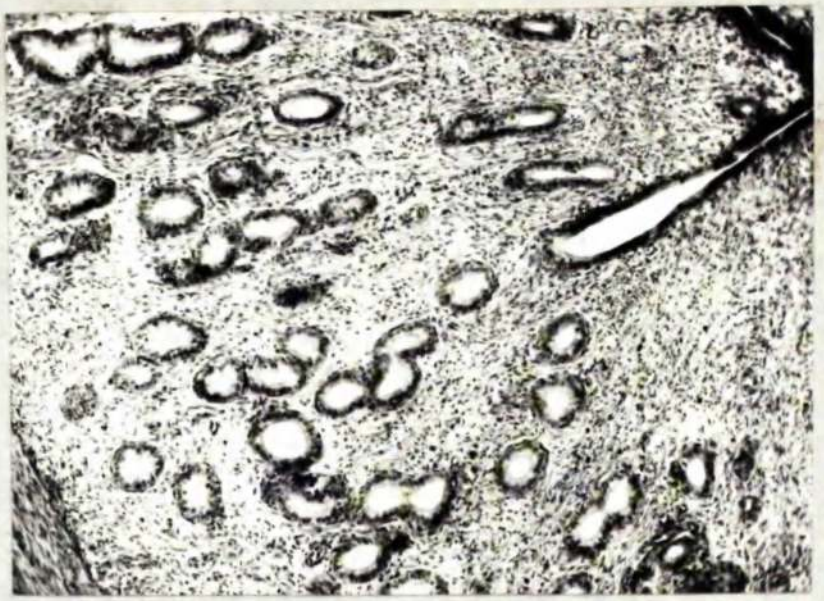

Fig. 61. Case D32: slightly dilated glands: lined by columnar cells: no secretion in lumina. H. and E. x 110.

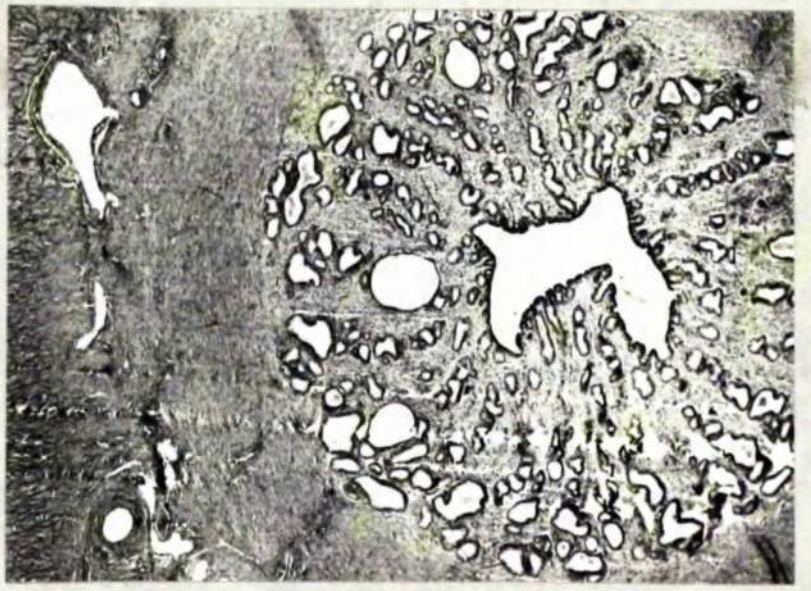

Fig. 62. Case D34: cystic glandular hyperplasia of endometrium. H. and E. x 35.

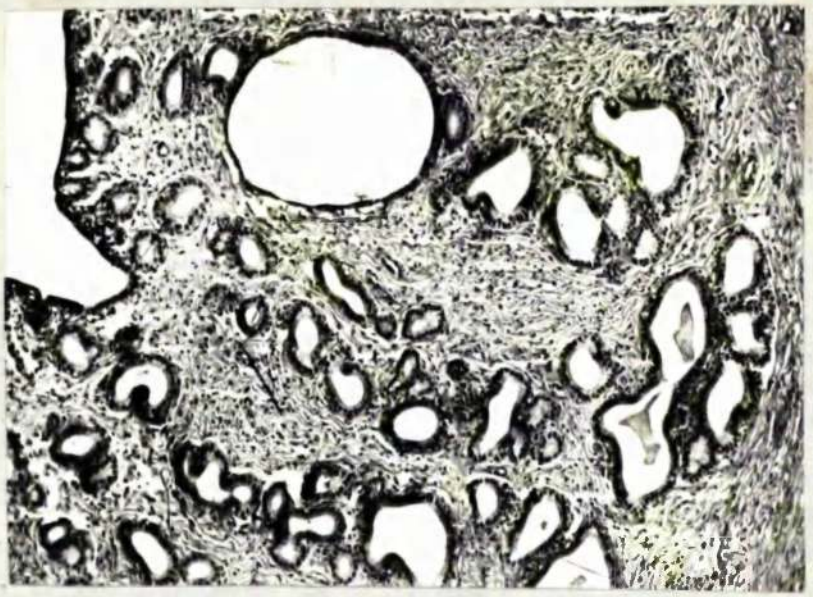

Fig. 63. Case D34: variation in cyst size: increased collagen deposition. H. and E. x 110.

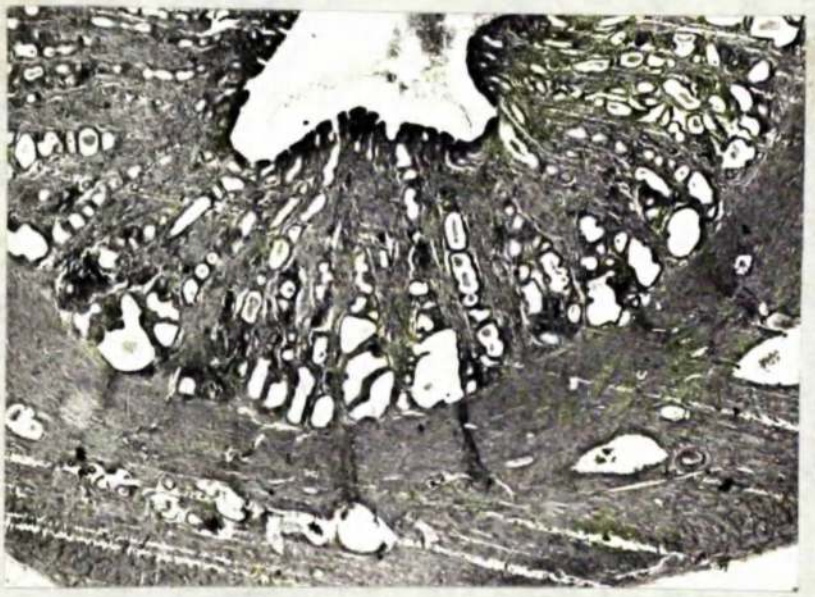

Fig. 64. Case D36: cystic glandular hyperplasia of the endometrium. H. and E. x 35.

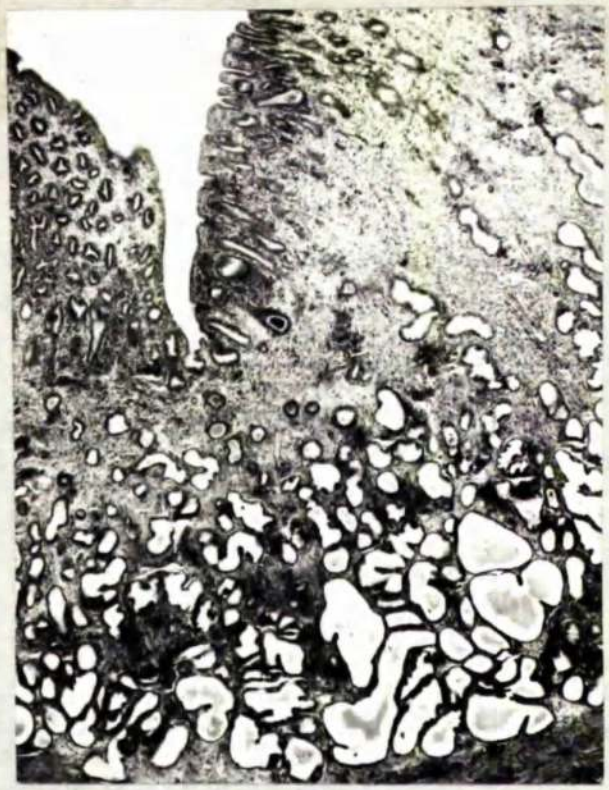

Fig. 65. Case D37: cystic glandular hyperplasia: dilatation of entire tubules. H. and E. x 35.

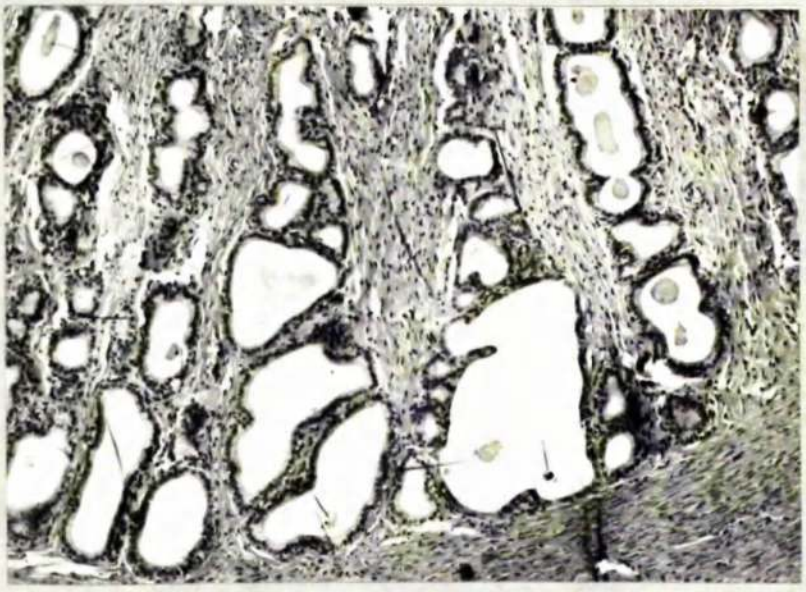

Fig. 66. Case D36: basal cyst: irregular shape: increased collagen deposition. H. and E. x 110.

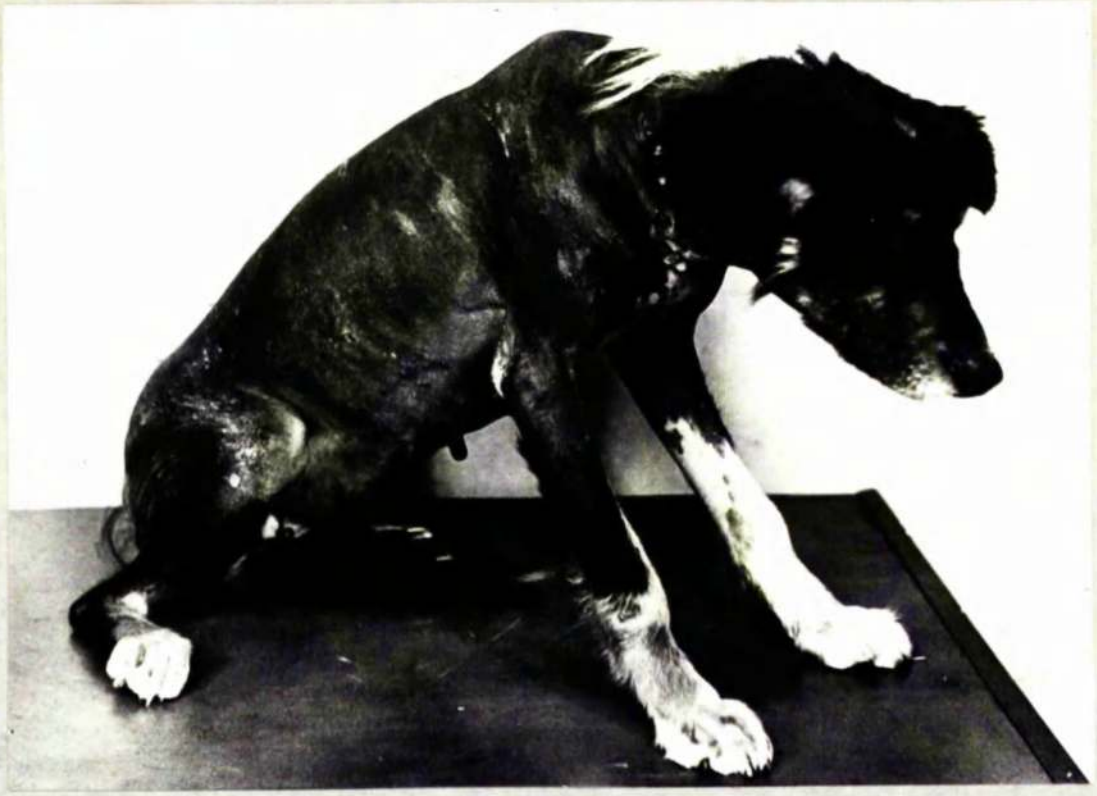

Fig. 67. Case D39: loss of hair following prolonged treatment.

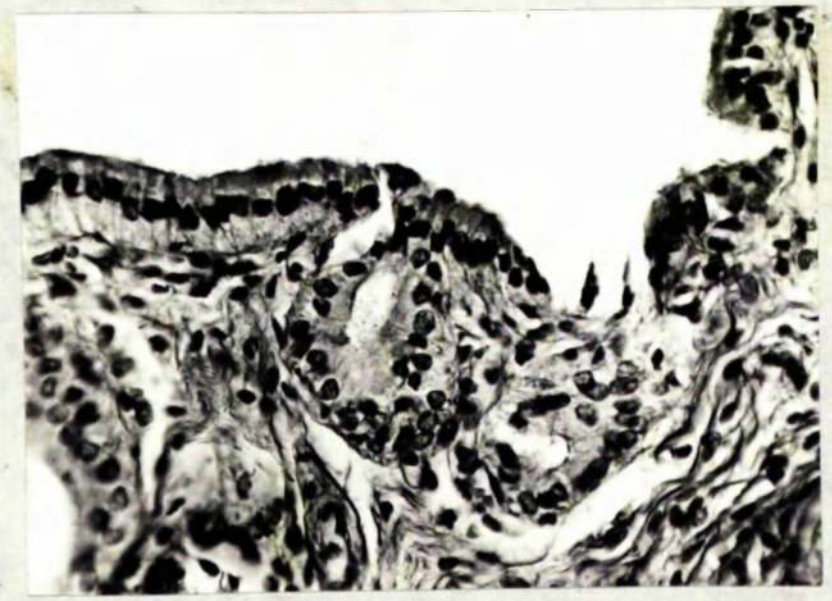

Fig. 68. Case D39: tall superficial epithelium with submuclear and supramuclear vacuolation. H. and E. x 400.

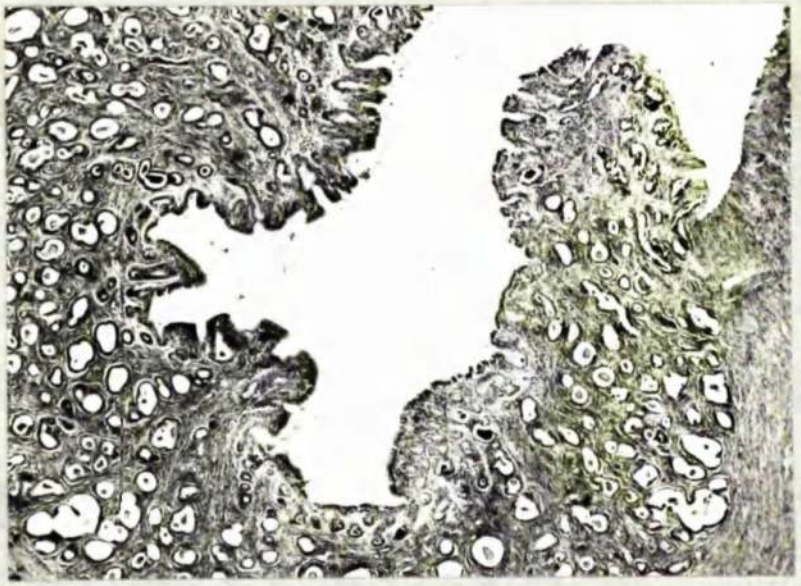

Fig. 69. Case D39: cystic glandular hyperplasia of the endometrium. H. and E. x 35.

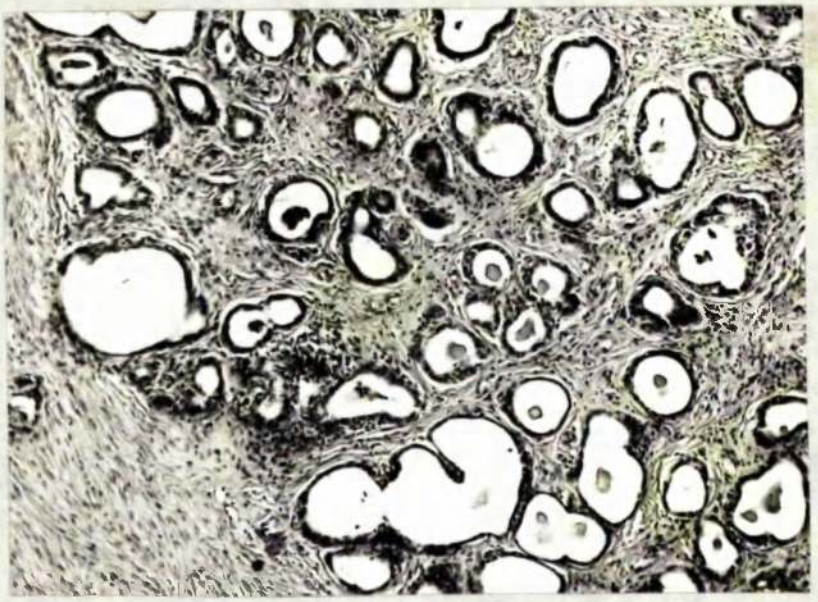

Fig. 70. Case D39: radial orientation of collagen fibres: variation in shape and size of cysts. H. and E. x 110.

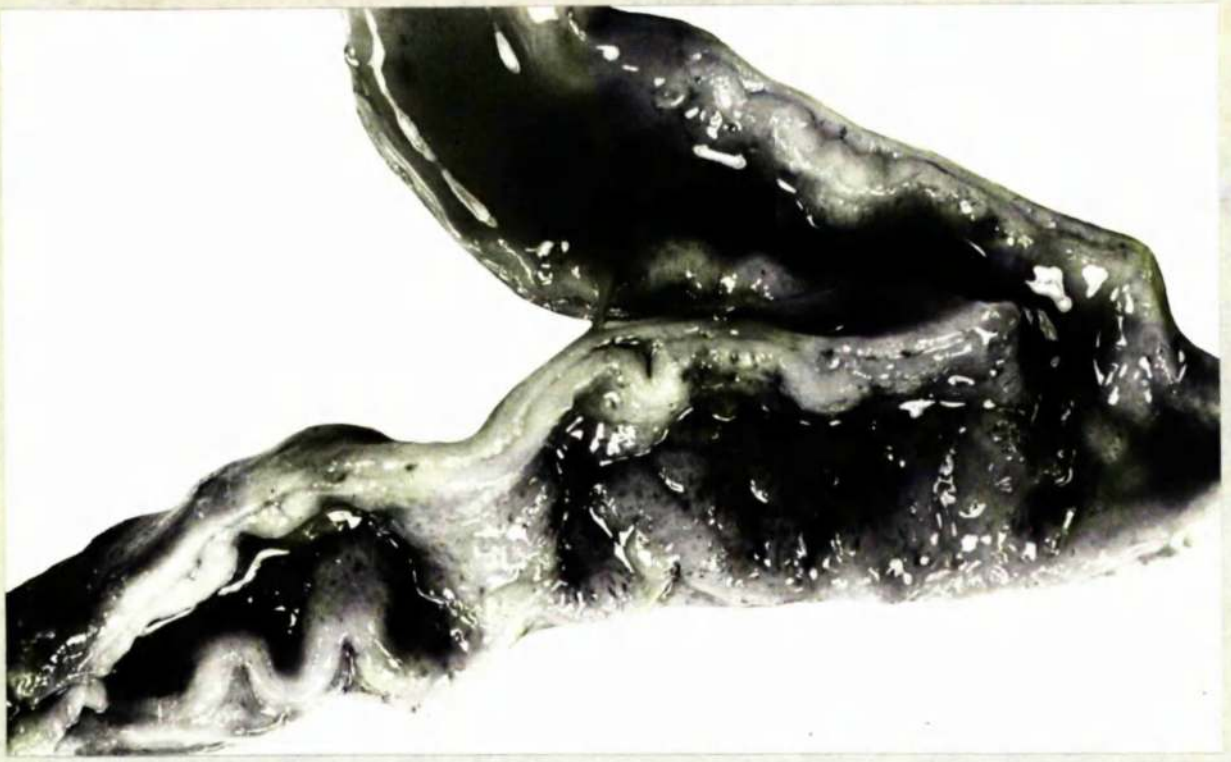

Fig. 71. Case D4.0: roughened endometrium: mucopus in left horn.

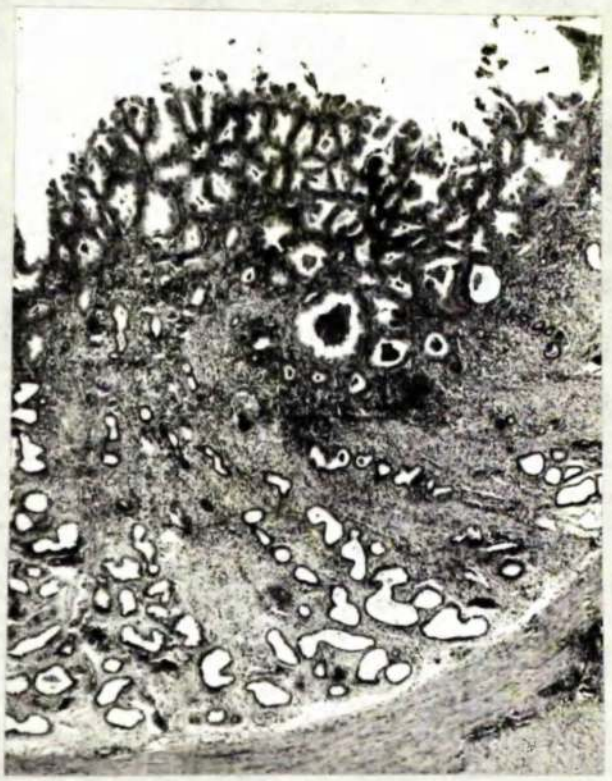

Fig. 72. Case D4.0: cystic glandular hyperplasia: acute endometritis. H. and E. x 30.

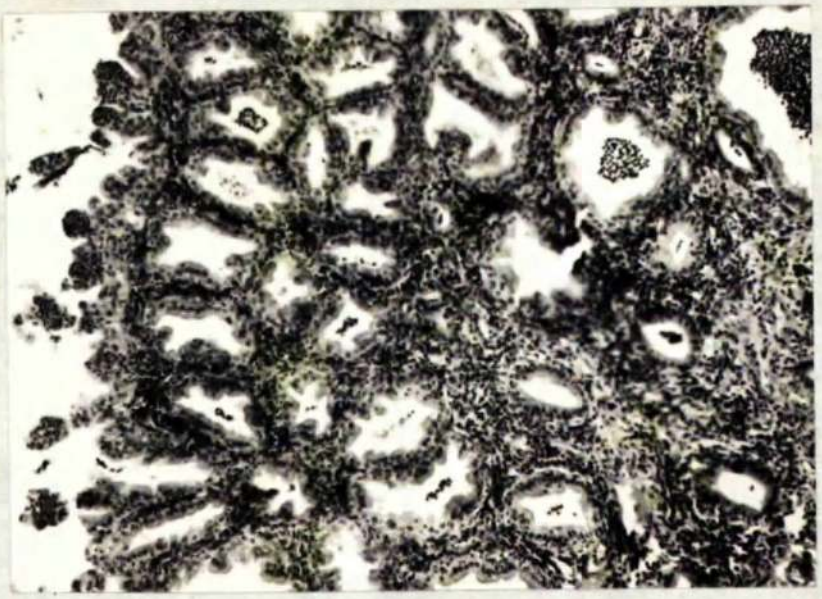

Fig. 73. Case D40: superficial cysts with neutrophils in the lumina and surrounding stroma. H. and E. x 110.

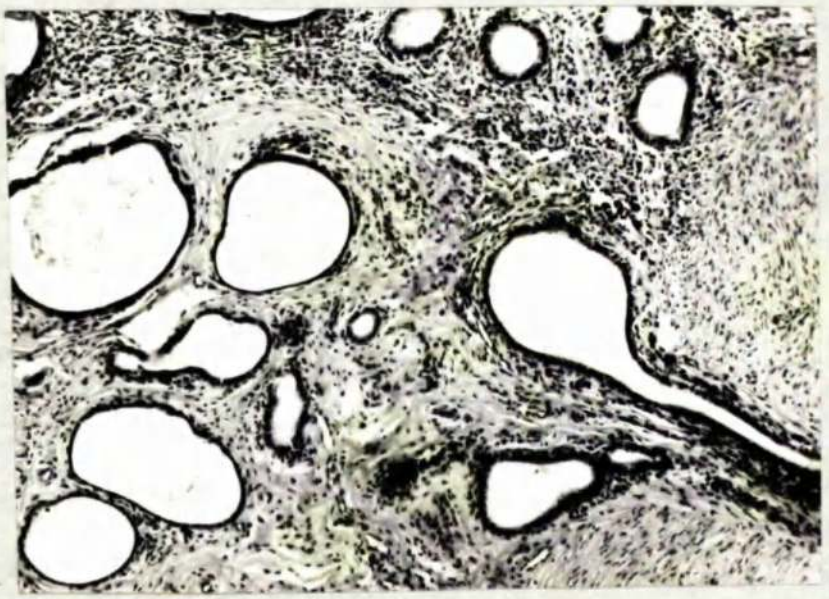

Fig. 74. Case D40: basal cysts lined by pavement epithelium: dense peri-glandular collagen. H. and E. x 110.

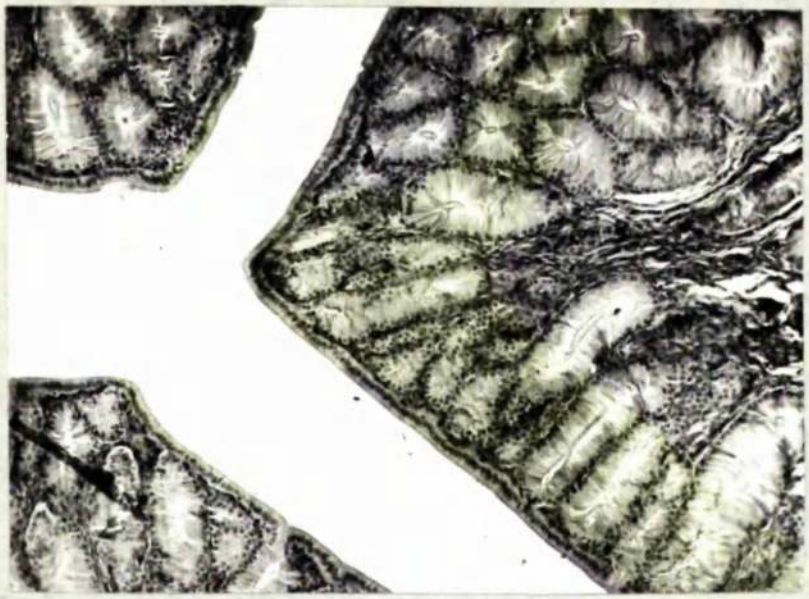

Fig. 75. Case D43: increased width of crypts: marked hypertrophy of lining epithelium. H. and E. x 110.

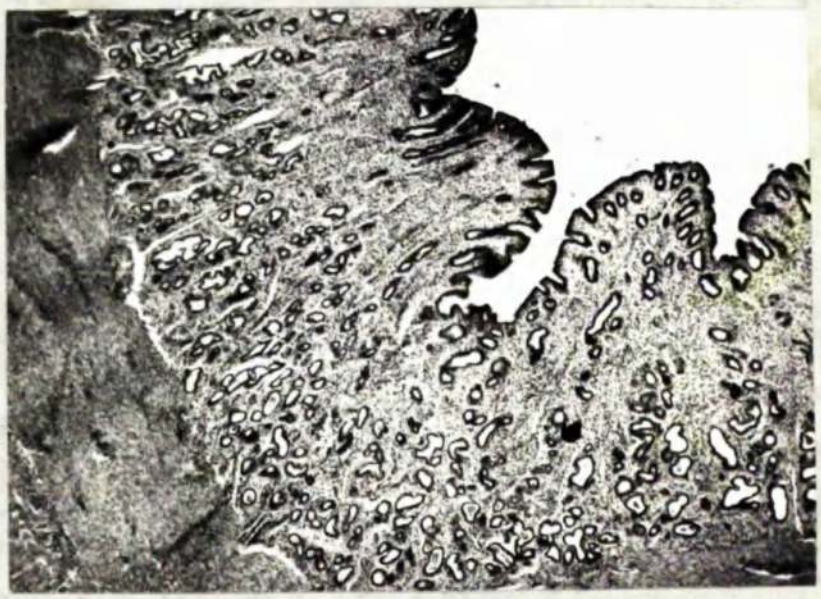

Fig. 76. Case D44: swelling of endometrium: mild dilatation of basal glands. H. and E. x 35.

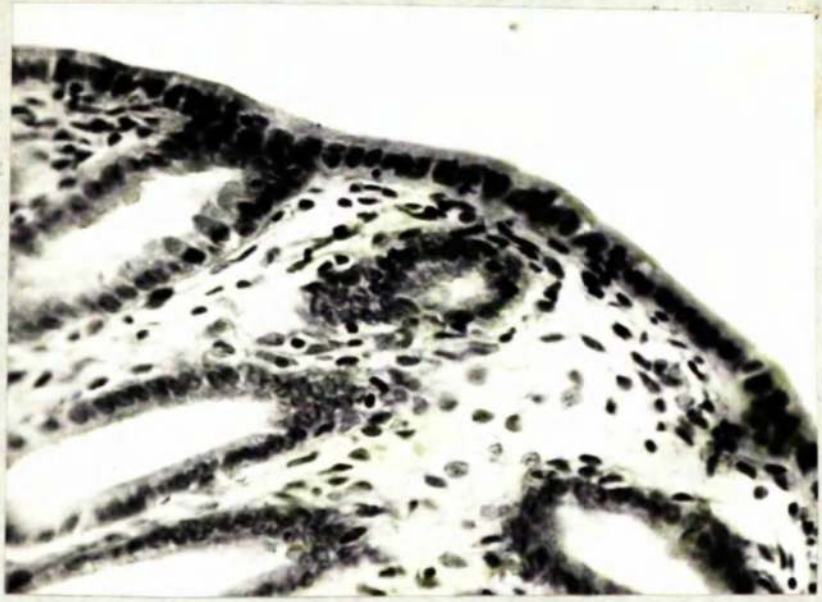

Fig. 77. Case D44: tall columnar epithelium of the crypts and surface: no secretion in lumen. H. and E. x 400.

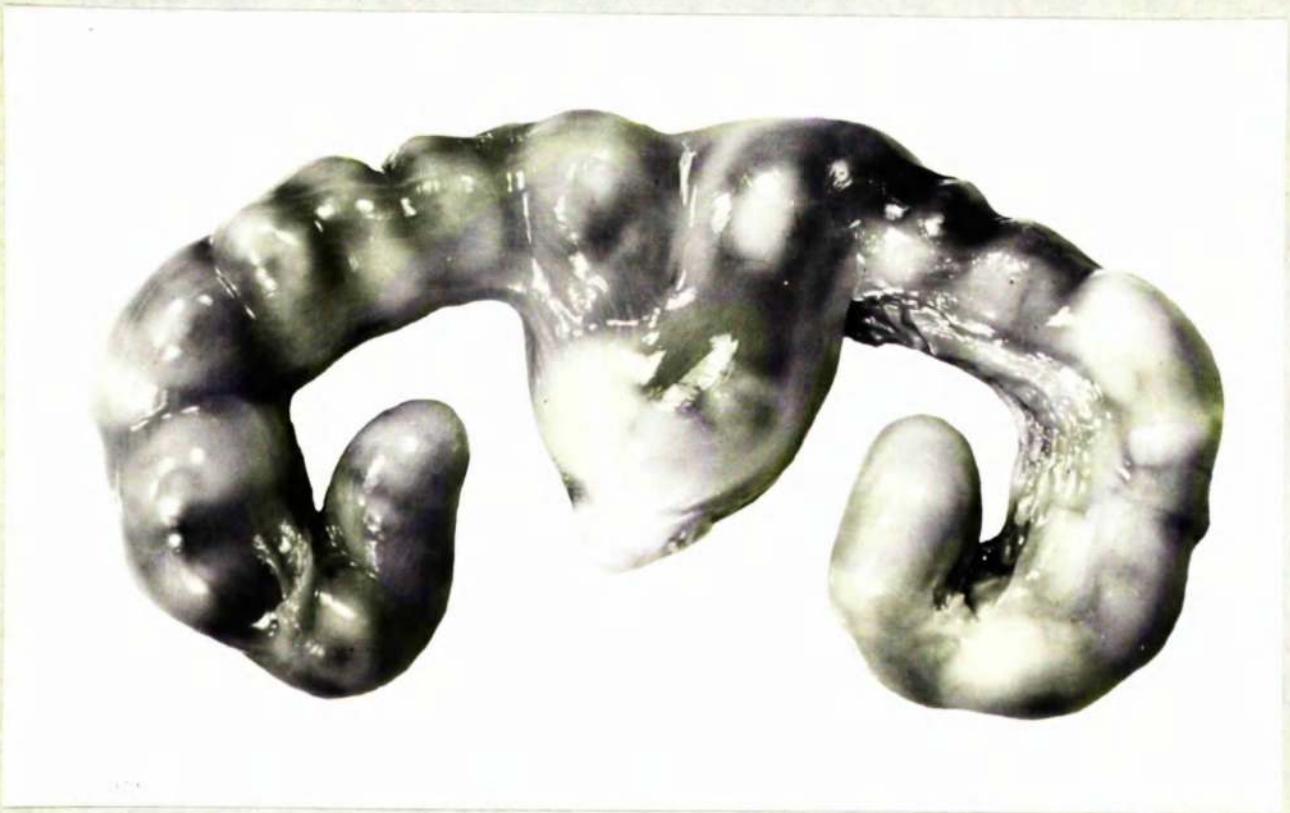

Fig. 78. Case D47: acute endometritis: ampullations of the horns.

Fig. 79. Case D47: endometrium showing acute inflammatory reaction.

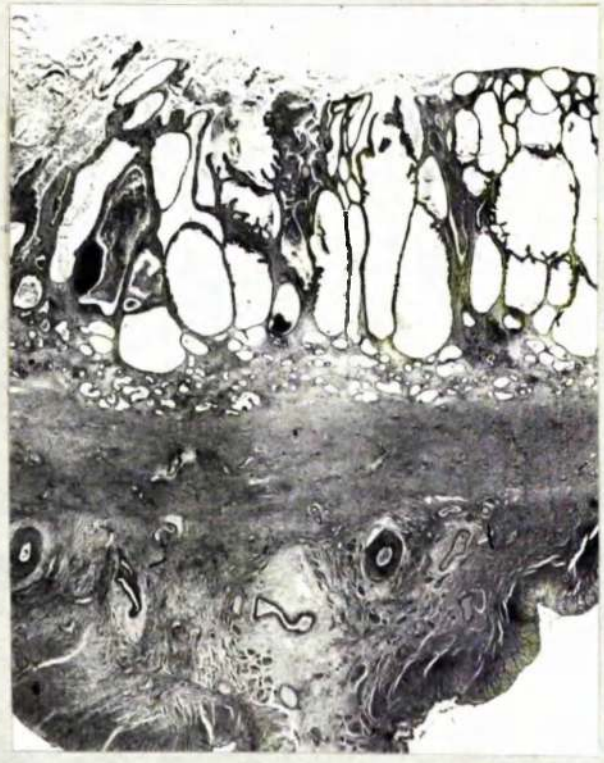

Fig. 80. Case D48: marked cystic glandular hyperplasia: mild acute endometritis. H. and E. x 10.

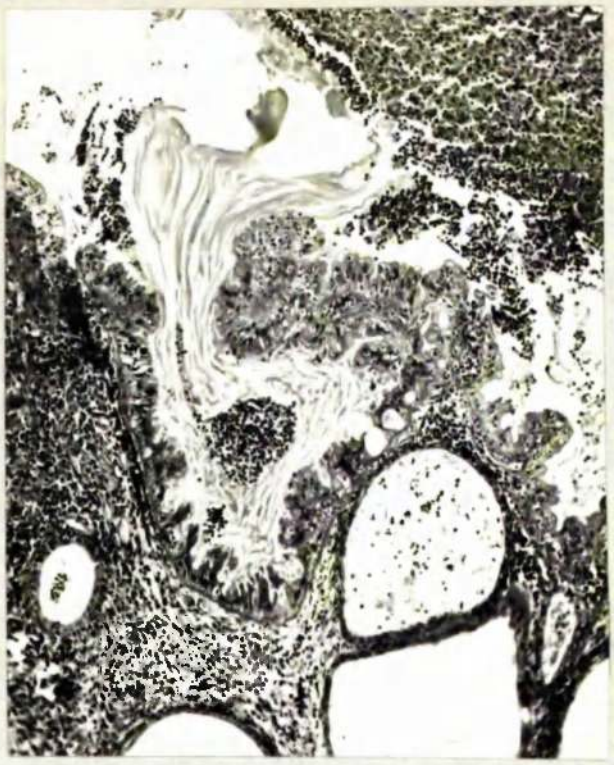

Fig. 81. Case D48: irregular proliferation of surface epithelium: intra-epithelial cysts: profuse secretion: diffuse infiltration by neutrophils. H. and E. x 110.

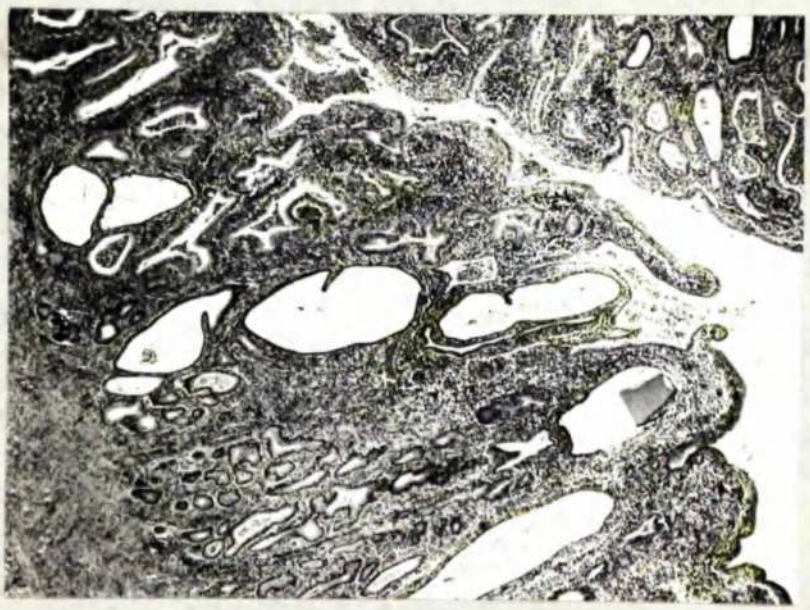

Fig. 82. Case D47: cystic glandular hyperplasia: acute endometritis. H. and E. x 30.

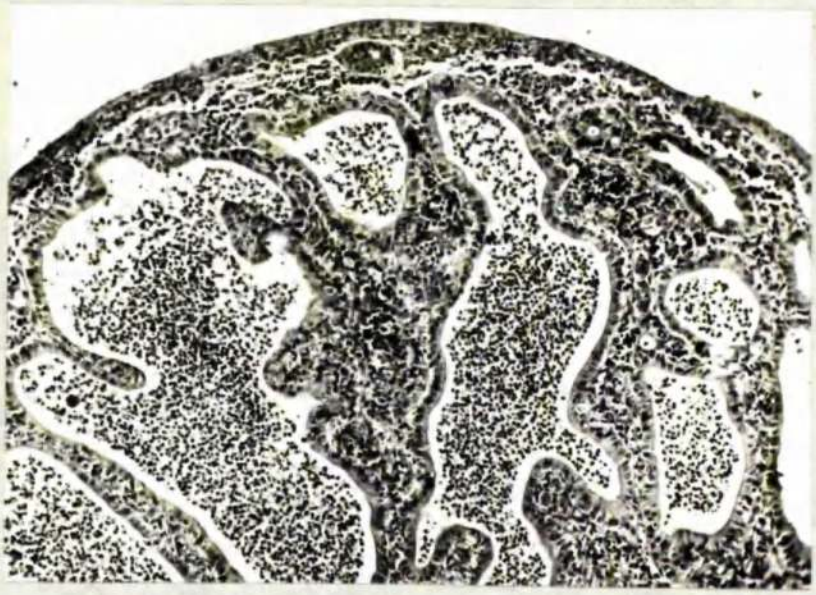

Fig. 83. Case D47: superficial cysts: diffuse neutrophil infiltration. H. and E. x 110.

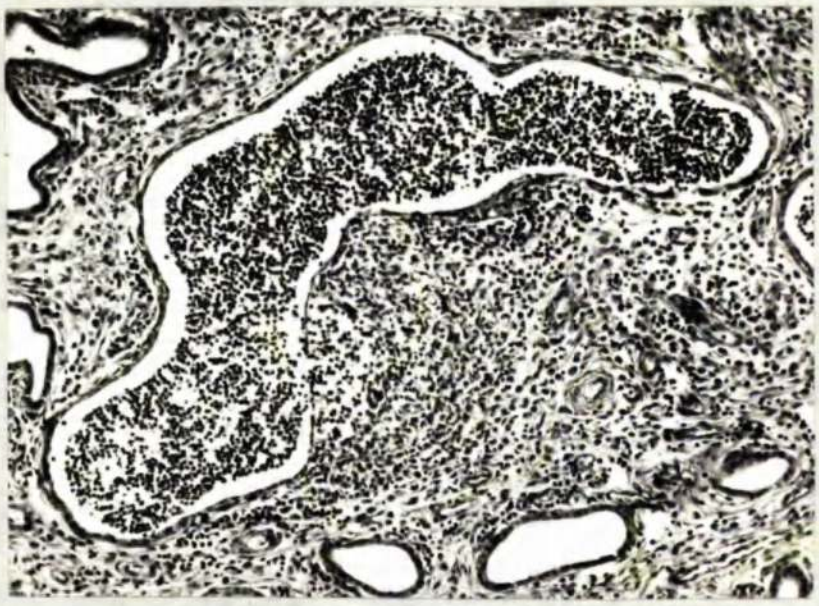

Fig. 84. Case D47: early abscess formation around blocked cyst. H. and E. x 110.

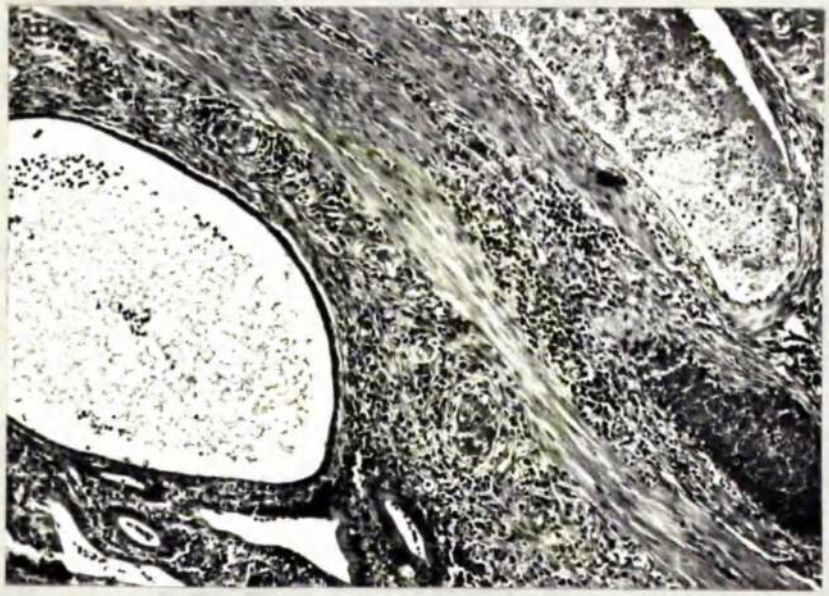

Fig. 85. Case D4.7: infiltration of the inner myometrium by inflammatory cells. H. and E. x 110.

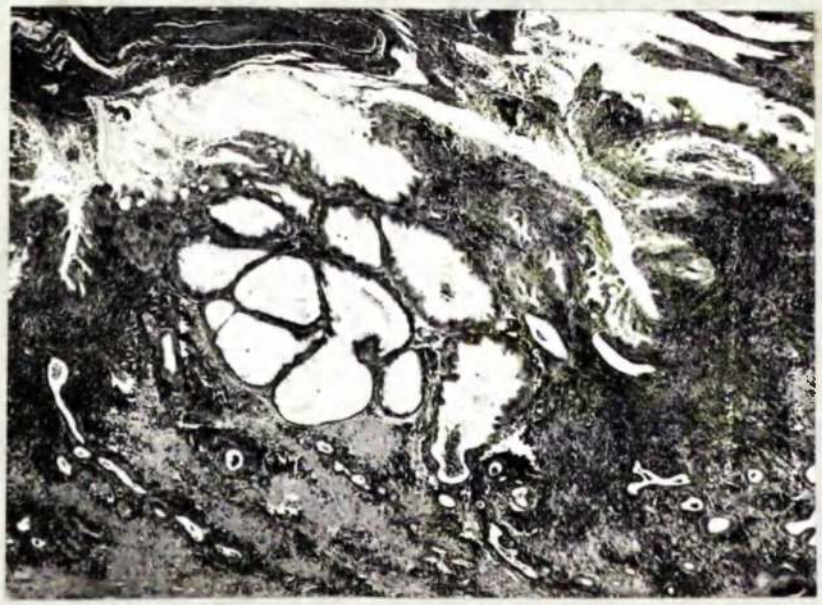

Fig. 86. Case D4.6: cystic glandular hyperplasia: severe acute endometritis. H. and E. x 35.

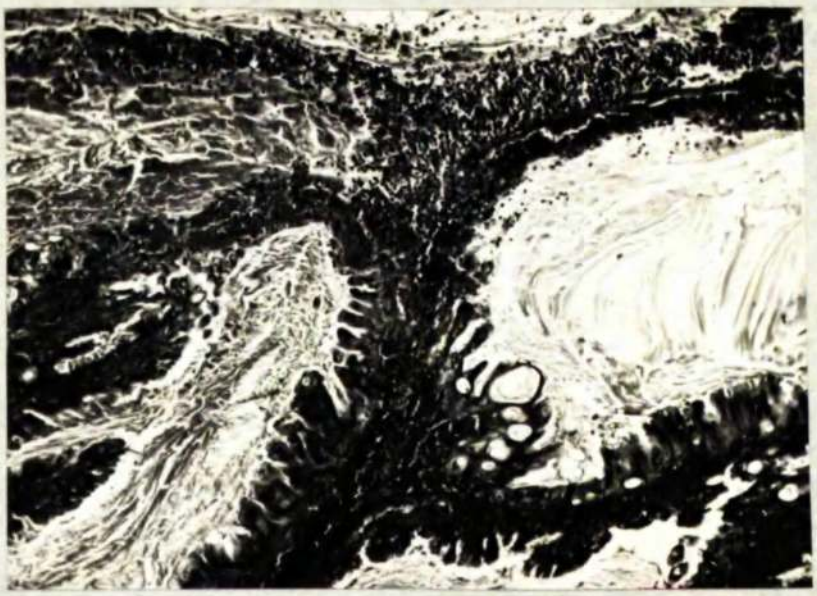

Fig. 87. Case D46: superficial ulceration of the endometrium: irregular proliferation of cyst epithelium. H. and E. x 110.

Fig. 88. Case D51: cystic glandular hyperplasia: cellular infiltration of crypt zone. H. and E. x 35.

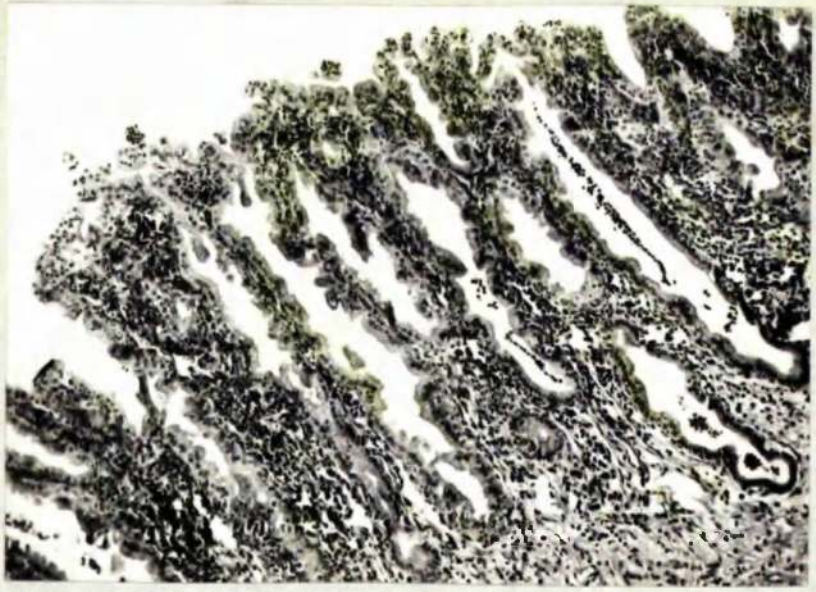

Fig. 89. Case D51: prolongation of crypts to form slender polypi: diffuse plasma cell infiltration: some neutrophils in lumina. H. and E. x 110.

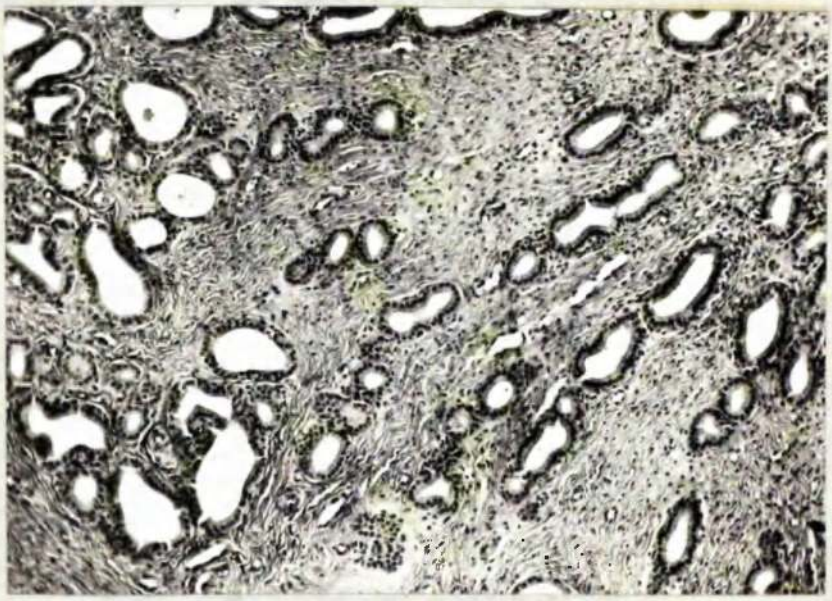

Fig. 90. Case D49: mild glandular dilatation : radial arrangement of collagen fibres. H. and E. x 110.

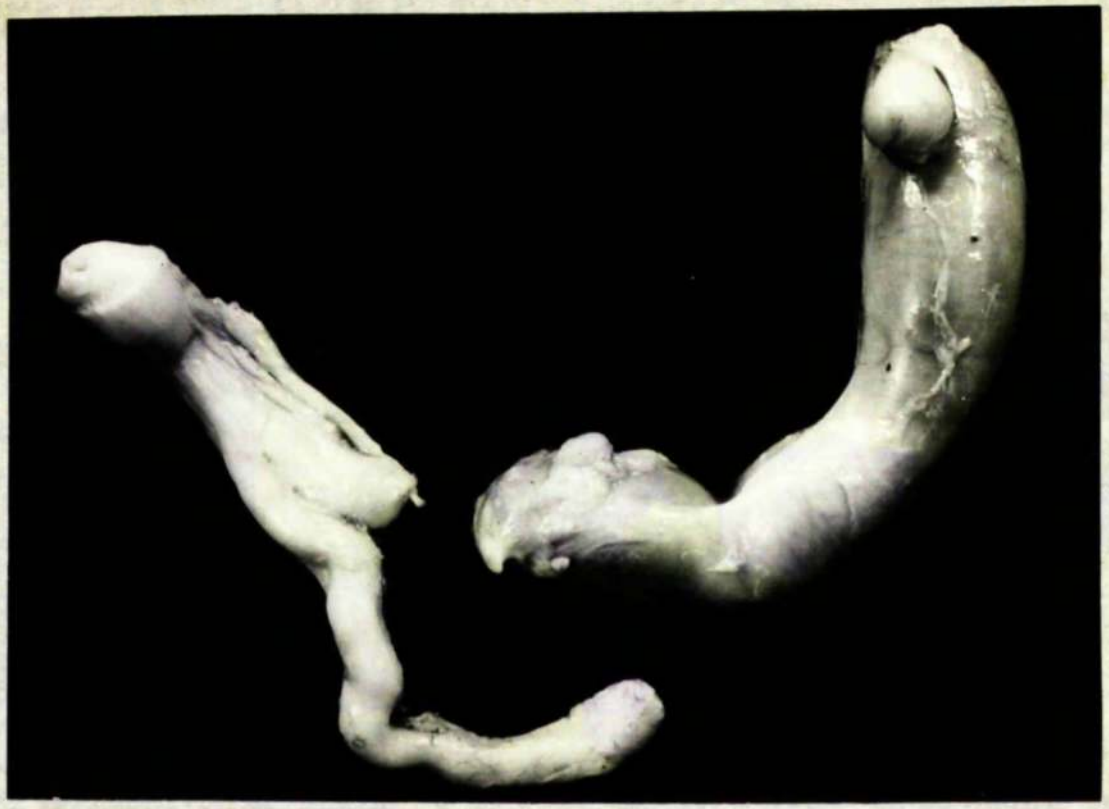

Fig. 91. Case D53: distension of isolated horn.

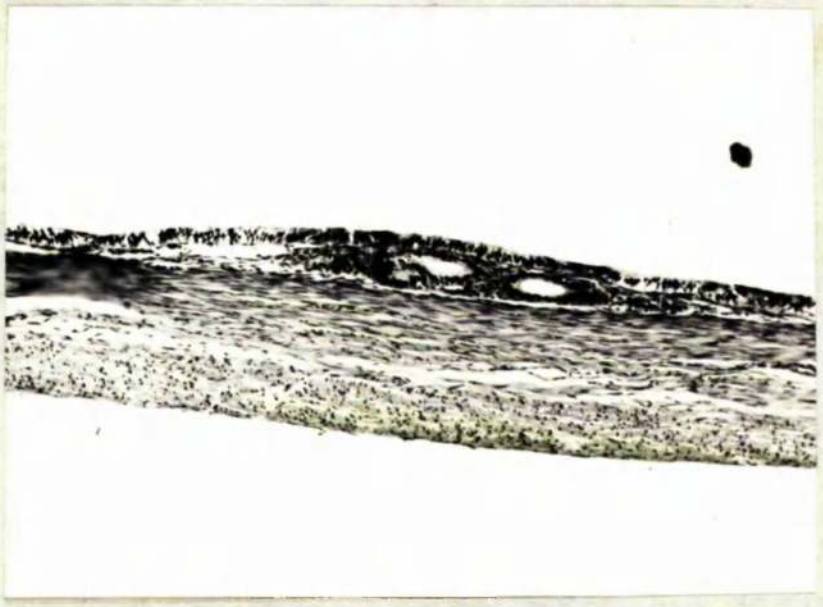

Fig. 92. Case D53: thinning of all strata of the endometrium. H. and E. x 90.

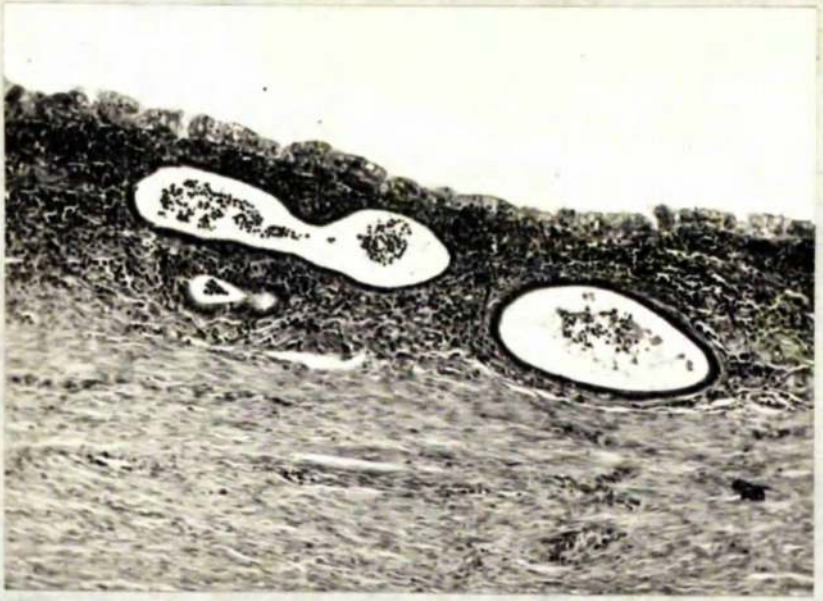

Fig. 93. Case D55: thinning of endometrium: stromal cells composed of neutrophils and round cells. H. and E. x 110.

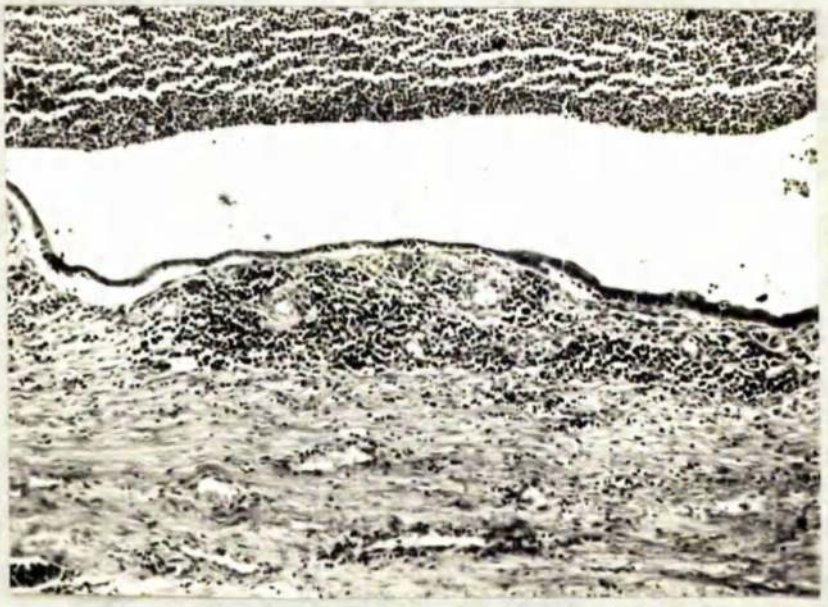

Fig. 94. Case D56: thinning of endometrium: stromal cells are mainly lymphocytes and plasma cells. H. and E. x 110.

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