STUDIES

on the

BACTERIOLOGY & CHEMISTRY

of the

VAGINA.

by

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C O N T E N T S.

CHAPTER I.

INTRODUCTION.

The bacteriology of the vagina has been the subject of much investigation and study for many years, the earliest important landmark in this work having been Döderlein's classic publication "Das Scheidensekret" in 1892. During recent years, intensive research on the causation of puerperal fever has further focussed attention on the vaginal flora. So much work has been done, in fact, on the bacteriology of the female genital tract, most of it certainly in the obstetrical field, that it would almost seem necessary that good reason should be adduced for adding to the enormous literature which has already accumulated.

Certain considerations have now made desirable a study of the chemistry and bacteriology of the virginal vagina. Firstly, in very recent years new conceptions have arisen of the close relationship between ovarian function and the biology of the vagina. Secondly, colorometric methods of accurately estimating the pH concentration (reaction) of small quantities of fluid have been simplified and have been brought well within the scope of clinical applicability. Thirdly, the clinical/

clinical problem of leucorrhoea in virginal women has induced one to review and investigate the whole subject of normal and abnormal vaginal secretion.

My studies have not concerned the secretion of the vagina during pregnancy or the puerperium and, accordingly, very little reference has been made to the literature in this field. The work essentially concerns "Leucorrhoea in Virgins" and the material employed has been, with few exceptions, the new-born, very young infants, children and adult virgins.

The relevant literature has been fully reviewed and the data - literature and original work - have been arranged in the following way:

(1) A review is first given of the knowledge of the bacteriology of the vagina in the adult, without reference to the bacterial flora in pregnancy.

(2) This is followed by an account of the vaginal epithelium and its contained glycogen. The present knowledge of the function and distribution of this substance in the epithelium and its relationship to the chemistry of the secretion is discussed.

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An account of the ovario-vaginal mechanism, involving ovarian control of vaginal epithelium and secretion is given. Original work, involving histological studies of glycogen in vaginal epithelium, from foetal life to the menopause, is then described and illustrated and the results discussed.

(3) The next portion of the work concerns the vaginal secretion from birth to puberty. The imperfect present state of our knowledge is indicated. There then follows an account of original work into the bacteriology of the vagina in infants and children and into the reaction (pH concentration) of the secretion. "Vaginal Haemorrhage in the New-born" is discussed.

(4) This is followed by an account of "Virginal Leucorrhoea." This condition and its possible causes are described in great detail. Original work, involving about 40 cases, studied over a period of three years, is then detailed. Based on the results of investigation of these cases and/

and on the findings described earlier in this work, a solution of the problem of the etiology of leucorrhoea in virgins is presented.

Acknowledgments.

I am greatly indebted to many people for having made these studies possible; firstly, to Dr. Robert Cruickshank, who has not only supervised and controlled the entire laboratory work, but has placed the facilities and material of the Bacteriology Department of Glasgow Royal Infirmary at my disposal; to Dr. Amy Fleming, who has afforded me facilities and material in the Pathological Department of the Royal Samaritan Hospital for Women, Glasgow; to Professor Munro Kerr and Dr. Stanley Graham for access to cases in the Royal Maternity Hospital, Glasgow; to Mr. Matthew White and Dr. Blacklock for clinical and pathological material, respectively, in the Royal Hospital for Sick Children; to Professor Shaw Dunn for advice in technique and opinions on my results of glycogen staining; to Dr. D. P. Cuthbertson for basal metabolism and blood-sugar/

blood-sugar estimations; to Dr. Bruce McLean for radiography of the sella turcica; and to the Visiting Surgeons of the Royal Samaritan Hospital for access to cases of virginal leucorrhoea. Finally, I must acknowledge my indebtedness to the McCunn and Carnegie Trustees who, by grants of scholarships, during the past three years, have materially encouraged these investigations.

CHAPTER II.

THE BACTERIOLOGY OF THE ADULT VAGINA.

The earliest studies of any significance on the vaginal flora were those of Gönner in 1887 and Döderlein in 1887 and 1892. These investigators were mainly concerned with the relationship of the flora to the development of puerperal sepsis. Gönner examined specimens of secretion and obtained pure cultures of cocci. Only rarely was he able to obtain cultures of the characteristic vaginal bacilli and he was unable to explain this. Experimentally, he showed that neither the cocci nor bacilli were pathogenic. Samschin's results in 1890 were similar. Döderlein stated that Bumm and Bockhardt had corroborated Gönner's work. As early as 1870 Haussmann had observed the bacilli. Winter (1888) realised the importance and value of these organisms to the vagina. Steffeck (1890) corroborated Winter's studies in most respects and observed that in the genital canal of healthy unexamined women, staphylococci and streptococci were present./

present. Döderlein's work marked a great advance at this stage. He stated that the vaginal secretion of pregnant women could be divided into two categories, (1), normal, and (2) pathological, on the grounds of naked-eye appearance, bacteriology and cytology, and reaction to litmus paper. In the normal cases, the secretion was highly acid in reaction and contained large numbers of the vaginal bacillus, some yeasts and practically no other organisms. In the other cases, the secretion was slightly acid, neutral or alkaline, and showed a mixed flora with absence of the characteristic vaginal bacillus. His findings were corroborated by Krönig (1894) and others, although several workers, e.g. Bergholm (1902) and Stolz (1903) disputed his conclusions on the significance of the presence of pathogenic organisms. Subsequent investigators were unable to agree that the vaginal secretion of all women could be clearly differentiated into Döderlein's two groups - (1) normal and (2) pathological.

Schröder (1921) classified all types of vaginal secretion into three groups or "grades of cleanliness," ("Reinheitsgrad"). Mayer in 1929 in

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a paper on the "Clinical Use of Vaginal Spreads" described three grades of contamination:

(1) The fully-normal virginal vagina contains almost exclusively the short, thick Gram-positive, blunt vaginal bacillus (in pregnancy longer and coarser — Grade 1).

(2) The second grade in addition to vaginal bacilli, contains other bacteria with or without pus.

(3) The third grade contains no vaginal bacteria, but one or more other varieties of bacteria with or without pus.

Cruickshank and Baird (1930) distinguished the three groups in pregnancy as follows:

(1) Grade 1. - vaginal secretion is tough, milk-white, highly acid (pH 4.3 to 4.9) and contains a few epithelial cells and very numerous Döderlein's bacilli;

(2) Grade 2 - secretion thinner in consistency, colour yellowish or greenish white, pH 4.6 to 5.0, some leucocytes are present and diphtheroids and cocci are present in considerable numbers with few Döderlein's bacilli;

(3)/

(3) Grade 3 - reaction is pH 5.2 to 6.8, plates of epithelium and many leucocytes are present, Döderlein's bacillus is absent, streptococci, coliform organisms, staphylococci, diphtheroids, yeasts and sarcinae are present, with Trichomonas vaginalis occasionally.

Cruickshank and Cruickshank (1931) point out that the figures obtained by various workers depend to some extent on the technique employed in taking the vaginal swabs. Special apparatus has been devised for this purpose by some investigators (Krönig, 1894; Lockhart, 1925). Contamination of the swab, by contact with the hymen, for instance, may convert a Grade 1 preparation into a Grade 2. With ordinary care, however, it is generally not difficult to obtain an uncontaminated vaginal swab.

The following photomicrographs illustrate the appearances of the grades of the flora:

Fig. 1. -
Grade I.
Photomicrograph,
oil-immersion.

Note -
Epithelial cell
and numerous
B. Döderlein.

Fig. 2 -
Grade II.
Photomicrograph,
oil-immersion.

Note -
B. Döderlein,
diphtheroids,
and cocci.

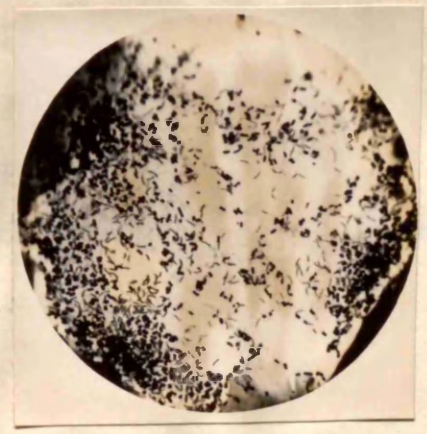

Fig. 3. -
Grade III.
Photomicrograph
oil-immersion.

Note -
Diphtheroids,
enterococci
and vibrios.

Since Döderlein's work in 1892, an enormous literature on the vaginal flora has accumulated. The following are a few of the authors whose publications have been of much importance and the date and the tenour of their papers are indicated alongside of their names:
Ahlfeld, 1893 (Auto-infection), Williams, 1893 and 1898 (Auto-infection and Importance of Vaginal Bacteria Clinically), Bumm, 1895 (Diphtheria), Gebhard, 1897 (B. coli communis), Dobbin, 1897 (Bac. aerogenes Capsulatus in Puerperium), von Rosthorn, 1899 (Puerperal Tetanus), Schottmuller, 1903 (Differentiation of Streptococci) and Noguchi and Kaliski, 1918 (Spirochaetes of Vagina in Health).

The pioneer work on the vaginal flora in pregnancy has been done by Döderlein, and by Krönig and Menge (1897). In normal pregnant women, bacilli of the Döderlein type, alone, have been found in fifty-six per cent. of cases by Cruickshank and Baird (1930) and in sixty-three per cent. by Logan (1931).

Winter,/

Winter, Steffeck and Döderlein described yeasts in the secretion in pregnant women and von Plautt (1887) identified them as Monilia candida. As early as 1877, Grawitz had described two forms of monilia development in the vagina, namely, conidia and mycelia. It was quite evident to many of these early workers that yeasts occurred more commonly in the pregnant state than otherwise and Döderlein attributed this to the increased acidity of the vagina during that time. (A full account of yeasts in the vagina is given later in this work under "Fungus Infections of the Vagina").

A full survey of the literature on aerobic and anaerobic streptococci in the genital tract is given by Schaffer (1917). He states that "the "organisms of the basic flora of the vagina are "all non-pathogenic, atoxic, in human cases "acidophilic, obligate and facultative anaerobic "saprophytes, that obligate aerobic organisms "seldom occur and that pyogenic bacteria occur "very rarely and only as a passing phase."

According to Loeser (1920), a perfectly normal/

normal vagina contains (1) a large excess of the group of vaginal bacilli which coagulate milk and produce acid in the media; among these is the Döderlein bacillus, Bacillus vulgaris and Bacillus ordinarius: (2) Pseudo-diphtheria bacilli: (3) Saccharomyces: (4) Bact. coli: (5) Bact. acid lactici: (6) Bacillus acid lactici, comma variabile, Bacillus thetoides and various types of cocci including staphylococci. Yeasts may be found. In a perfectly healthy vagina, only vaginal bacilli and comma variabile are found in the smear. In the presence of contamination, more and more of the other bacteria appear.

The changes which occur in the vaginal secretion during and after menstruation have been studied by Demme and Baltzer (1927). During the menstrual flow, the washing with blood and tissue fluids alters the growth relationships of the bacteria of the vagina, but Döderlein's bacillus is soon re-established as soon as menstruation ceases. An extensive study of the vaginal secretion has been made by Schröder, Hinrichs and Kessler (1926)./

(1926). These authors have given detailed descriptions of the morphology and cultural characters of the vaginal bacillus and have described marked changes in morphology according to the pH value of the medium on which it is grown.

The vaginal bacillus is of such paramount importance in these studies that it is essential that its characteristic features should be described. The description by Cruickshank and Cruickshank in the "System of Bacteriology" (1931), published by the Medical Research Council, is freely quoted. Döderlein (1892) described the occurrence in the vagina of healthy pregnant women and virgins of a long, Gram-positive, non-motile bacillus which grew aerobically or anaerobically on glucose agar as translucent dew-drop colonies. It came henceforth to be recognised and described as the Döderlein bacillus. He found that large quantities of lactic acid were formed by it and that in mixed cultures with staphylococci, the latter were rapidly killed by the resulting acid condition. The lethal action of the vaginal secretion/

secretion was demonstrated by the experimental introduction of staphylococci into the vagina of a virgin. Döderlein realised that the secretion in the vagina of the new-born was acid before bacilli had yet appeared, but attributed this to carbonic acid, believing that lactic acid did not appear until bacilli were present. Menge (1897), however, showed that the property of eliminating organisms artificially introduced into the vaginal canal was independent of the acidity of the vaginal secretion, as similar self-cleansing powers were present in women whose secretions were neutral or alkaline. He maintained that vaginal bactericidal action was due to lack of oxygen, to leucocytic and to bactericidal tissue juices. Krönig (1894) stated that the vagina of newly-born children and virgins showed a similar power of disinfection. The morphological resemblance of Döderlein's bacillus to the lactic acid bacilli has led, during recent years, to its classification with the group of Gram-positive aciduric bacilli present/

present in the intestine (Schweitzer), 1913, and others). Jötten (1922) asserts its identity with B. acidophilus. Its resistance to highly acid conditions of culture has been shown to be considerable (Demme, 1927; Miura, 1928). A direct smear taken from the vagina during the later months of pregnancy and stained by Gram's method, shows in a large proportion of healthy women an almost pure growth of a slender Gram-positive bacillus, straight or slightly curved, occurring singly, in pairs or in small bundles. Shorter, stouter forms may be seen, but it is difficult to decide whether these represent true Döderlein bacilli or are diphtheroid organisms which are frequently present in the flora. Films from culture show the same type of organism. Different strains show considerable pleomorphism in cultures: there are short and long, thick and thin forms, occurring singly or in pairs or parallel rows, chains of bacilli or cocco-bacilli and, particularly in older cultures, long thread-like and involution forms. (Jötten, 1922). The bacillus is non-motile, non-sporing and uncapsulated./

uncapsulated. Although anaerobic conditions are most favourable for its primary isolation, the bacillus once isolated grows readily in the presence of oxygen. Serum agar and blood-agar have been found to be good media for its isolation and growth. Rother (1922) has found that serum and ascitic fluid enhances growth only so far as these fluids contain glycogen and further, that glycogen cannot be utilised by the organisms until it is converted to sugar by enzyme action. It is a powerful producer of acid, forming as much as 150 c.cs. of normal acid per litre of glucose broth. The following photomicrographs illustrate features of Döderlein bacilli:

Fig. 4 - The commonest type; photomicrograph, oil-immersion.

Fig. 5 - Long chains, much less common; photomicrograph, oil-immersion.

Fig. 6 - Rare form with bulbous swellings; photomicrograph, oil-immersion.

This brief review of the bacteriology of the vagina indicates its main features and the essential landmarks in our progress to the present-day conceptions of the classification of the flora in terms of "grades of cleanliness." On this basis, rather than on that of the precise recognition of individual organisms, rests the study of the flora of the vagina as applied to clinical problems.

CHAPTER III.

GLYCOGEN IN THE VAGINAL MUCOSA, AND ITS RELATIONSHIP TO THE CHEMISTRY OF THE SECRETION.

Structure of the Mucosa — Review of Knowledge of Glycogen and Lactic Acid in Vagina — Ovario-vaginal Relationship — Original Investigations into Glycogen in the Epithelium.

Structure of the Mucosa.

Most of the important modern text-books describe the histology of the vaginal wall more or less identically, but very few of them make any reference to the presence of glycogen in the epithelial cells. It would appear that the significance of this substance, present so abundantly in the cells, is not fully appreciated.

The vaginal epithelium is of the stratified squamous type, resembling skin; three layers of cells can be distinguished in it. The deepest layer consists of one or two rows of low columnar cells with single large nuclei./

nuclei. Above these lie several rows of typical "prickle" cells. Near the columnar layer these are small and closely packed together, but nearer the surface they are larger and more definitely polygonal. The superficial layers consist of flattened cells with few nuclei and indistinct outlines, i.e., cells that have undergone keratinoid changes. Many layers of degenerated cells may be seen in process of separation from the surface. Many of the cells in this layer appear to be vacuolated. The epithelium has a well-marked basement membrane, lying in immediate contact with the tunica propria, into which numerous, short, conical fibrous papillae extend. Cornification and pigmentation are not present except in pathological conditions where the mucosa is exposed to external irritation. The fibrous and elastic tissues of the tunica propria contain many veins and small lymphoid nodules and replaces the submucous layer. The muscular coat consists of two layers of unstriped muscular bundles, an inner circular and an outer longitudinal coat.

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The tissue surrounding this is made up of connective tissue, elastic fibres and many blood-vessels. Nerve fibres run in the musculature, giving off branches at right angles, which sub-divide and penetrate to the epithelium. The lymphatics of the upper two-thirds of the vagina form a fine network in the sub-epithelial connective tissue. They assemble to unite into two trunks which course upwards on to the posterior wall, join the superior haemorrhoidal vessels, surround the rectum and empty into the superior haemorrhoidal and mesorectal glands (Bruhus). The lower third drains into the inguinal glands. Lymph folicles occur particularly in the upper half of the vagina and lie beneath the epithelial layer.

The existence of vaginal glands has been much questioned. Frank states that Eppinger, Nagel, Gebhard, Pretti, Waldeyer, Williams and others deny their existence. He himself has never seen them in the examination of/

of a large number of specimens. Hennig, however, described them in 1870. Moreover, von Preuschen (1877) found definite glands in the vagina in four of thirty-six bodies which he carefully examined. The necks of these glands were lined by squamous epithelium, while the deeper portions, which spread out into definite bays, were lined by cylindrical epithelium on which cilia could be detected. Opening into the dilated glands were little crypts. Robert Meyer (1901) has described glands in the vagina of infants, and states that they are cervical in type, simple or compound tubular glands, being lined by ciliated cylindrical epithelium. Cullen, in 1905, stated that in the adult, vaginal glands are occasionally encountered and he described a small oval gland space in the vagina, lined by one layer of high cylindrical epithelium.

Nevertheless, glands that are found so rarely cannot be considered as a constant structure of the vagina (Frank, 1931). Most gynaecological pathologists classify the majority of these structures/

structures as aberrant cervical glands.

It is quite obvious, from the foregoing, that the vagina is not at all constructed in the manner of a secreting or glandular organ. Döderlein emphasized this and suggested that the word "secretion" was, strictly speaking, a misnomer. One must therefore seek an explanation for its peculiar discharge in some other mechanism. This is found in the constant and abundant shedding of cells, richly laden with glycogen, and the coincident production of large amounts of lactic acid. It is essential that we should review and summarise our knowledge of glycogen and lactic acid in the vagina, and endeavour to establish the thesis of the paramount importance of glycogen in the mechanism of normal and abnormal discharge, and its relationship to age and ovarian activity.

Review of Knowledge of Glycogen and Lactic Acid in the Vagina.

The marked acidity of the vaginal contents in most adult women has been recognised by/

by all observers. Its cyclical variation, involving an increase before and during menstruation, has been demonstrated by Gräfenberg (1918) and has since been confirmed. During pregnancy the acidity rises markedly and is at its highest point just before parturition. (Cruickshank and Baird 1930). The acidity is due to lactic acid which is present in a concentration of 0.4 per cent. (Becker 1909; Miura, 1928), and may rise during pregnancy to 0.9 per cent. (Harada, 1916). According to Ulrich (1931), the first attempts at microchemical estimation undertaken to estimate exactly the vaginal acidity date from 1926, from the publication of the work of Polish investigators, Zwozinski and Truszowski. They have found that in normal and in pregnant women the acidity varies from .4 to .65 per cent., that it is markedly diminished in infective conditions and averages .5 per cent. lactic acid. It is their view that the degree and intensity of the vaginal acidity is in direct proportion to the purity of the flora. Schröder (1930), Schultheiss (1929) and other authors state that/

that the reaction of the secretion in the normal healthy vagina varies from pH 4.5 to pH 6.2. It may be interpolated at this point that true neutrality is pH 7.0, alkalinity is pH greater than 7.0 and acidity pH less than 7.0: a very acid urine has pH 4.6 to 5.0 (approx.), normal urine around 6.0 and alkaline urine about pH 8.0.

Demme (1927) has shown that acidity of such a degree as is found in the normal vagina is of considerable importance in inhibiting the growth of the greatest majority of organisms. The pH limits of development for their growth in the usual culture media are as follows (Guillaumin, 1931):

	pH Limits of Development.
Gonococcus	6.0 — 8.3
Staphylococcus	5.6 — 8.1
Streptococcus	5.5 — 8.0
Pneumococcus	7.0 — 8.3
B. coli	4.4 — 7.8
B. pyocyaneus	5.6 — 8.0

B. acidophilus/

B. acidophilus, yeasts and diphtheroids are, of course, highly resistant to acid media. Döderlein's bacillus is now recognised as being a member of the acidophilus group of bacilli. It is capable of growth after days in a culture fluid of pH 4.0 (Schultheiss).

The presence of such large amounts of lactic acid in the vaginal secretion necessarily postulates a fermentable carbohydrate as a precursor (Cruickshanks) and some early workers suggested that glycogen is the substance concerned. (Döderlein 1892; Kronig and Menge 1897; Zweifel, 1908). Its presence in the cells of the functional layer of the epithelium has since been confirmed. Moser (1928) and Gisbertz (1930) have shown that it is not present in the basal layer or in the keratinised cells. It is, however, apparently uniformly distributed throughout the length of the vagina. In their high glycogen content the cells of the epithelium resemble hepatic, muscular or embryonic cells. The presence of glycogen

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is evidenced clinically by its staining with iodine in the course of vaginal preparation prior to operation.

It has been noted by Kienlin (1926) that lactic acid is present in the vagina of the newborn child before organisms have entered, and Miura (1928) has shown that, experimentally, glycogen is not fermented by cultures of the vaginal bacillus. Therefore it would appear that the lactic acid in the vagina is not due to the direct action of the vaginal bacillus upon the glycogen in the wall. Kienlin holds the view (as did Zweifel in 1908) that lactic acid arises through the breakdown of glycogen due to hydrolysis and that glucose and glucosamin are intermediate products. He maintains that vaginal bacilli do not play the essential part in producing lactic acid but that a fermentative process is undoubtedly at work. Loeser has also shown (1925), by estimating the lactic acid content (Warburg's method) of excised vaginal mucosa in Ringer solution, that this acid can be produced in large quantities without the action/

action of the bacteria. Again, it has been pointed out that, although a considerable amount of lactic acid is present in haematocolpos fluid, Döderlein's bacillus has never been demonstrated in that fluid. It would appear therefore, from these observations that there resides in the vaginal mucosa the intrinsic power of converting glycogen via intermediate products into lactic acid and that an enzymic or fermentative process is at work. A process, similar in part, has been shown by Claude Bernard to take place in the liver, namely, the conversion of glycogen into glucose by a special ferment. It is not disputed, however, that bacterial action may also play some part in the production of lactic acid. The intermediate products are probably the same, irrespective of the mechanism; von Jaschke (1925) points out that it is doubtful if any organism can convert glycogen into acid without some intermediate "splitting-up" of the sugar molecule.

The exact chemistry of the vaginal secretion has been studied in detail by Raab (1928)./

(1928). He states that it contains 2.7 per cent. albumen and products of protein autolysis. Of the amino acids, tyrosine, histidine and arginine are found. Tryptophane cannot be demonstrated and the ether soluble fraction of the xanthoproteic reaction is small. The secretion varies in pH from 4.2 to 6.4 and when acid the autolysis is due to pepsin-like ferments from the dead cells. When alkaline, the autolysis is like that of a peptidase and the enzymes come from leucocytes. Protein digestion by bacteria plays a subordinate role. The lactic acid of the secretion is an essential factor for intensive proteolysis. The urea is high and must be considered as a transudate. Raab thinks it probable that there is a current of fluid through the vaginal epithelium into the vagina.

Irrespective of possible ovarian factors which may control the deposition of glycogen in the vaginal epithelium, it must be assumed that this substance is being laid down as a result of conversion from glucose in the/

the blood-stream. Peters and Van Slyke, (1931), state that the liver is the only organ that can form glycogen from other material than glucose or polymers of glucose. There is no reason for believing that the formation of vaginal glycogen is intimately related to lactic acid in the blood. The only relative point of interest which I could garner from this field of study is that Bokelmann (1927) claims that blood lactic acid is high in normal pregnancy. His observations, however, are apparently not confirmed.

Since Zweifel's work in 1908 when he investigated in great detail the chemistry of the vaginal secretion and showed conclusively that the acidity of the vagina was not due to carbonic acid, which had been current opinion, very few important publications concerning glycogen in vaginal epithelium have appeared and these almost all by German workers. Loeser in 1920 demonstrated the important role of this substance in the protective mechanism of the vagina and its intimate correlation with the production of high concentrations of lactic acid. He showed that, in constitutional/

constitutional conditions where vitality is lowered there is a lessening in the functioning power of the epithelium, through loss of glycogen. He also suggested that ovarian dysfunction might be responsible for changes in the vaginal flora. Lehmann in 1921 corroborated Loeser's views and stated that the exfoliation of superficial vaginal cells, laden with glycogen, is essentially defensive. He stated also that the glycogen content of the vaginal epithelium goes parallel with the ovarian cycle and can be correlated with maturation of the follicle. At the time of the approach of menstruation and especially during pregnancy, the glycogen is increased. Changes in the bacterial flora were ascribed to the cyclical variation of the glycogen content of the vaginal epithelium. Corner, in 1923, in the course of an investigation into ovulation and menstruation in the monkey, Macacus Rhesus, described the presence of glycogen in both vaginal smears and in sections of vaginal wall. He was led to this study by the descriptions of Ascheim (1911) and of others of a cycle/

cycle of glycogen production in the uterus. They had demonstrated an increased quantity of this substance in the pre-menstrual uterus. Corner found that the freshly desquamated epithelial cells were laden with glycogen, as were the cells of the whole thickness of vaginal epidermis. He concluded that there is a more or less cyclic discharge of glycogen into the vaginal lumen, and that the quantity is greater during the latter half of the intermenstrual interval.

Schröder, Hinrichs and Kessler (1926) have published some detailed work of much importance. They state that the bacillus acidophilus grows in intimate symbiosis with the normal vagina. The glycogen is reduced to a monosaccharid and utilised by the bacteria to produce lactic acid, which in proper concentration kills off invading germs. Discharge from above (especially cervical) or massive introduction of bacteria from below, may overcome the protection. In the upper one-third of the vagina, the alkaline cervical secretion neutralises the vaginal/

vaginal acidity. Below this area other bacteria can flourish if the reaction is not below pH 4.6.

In a comprehensive study, Miura (1928) has shown that a direct association exists between ovarian activity, glycogen deposition in the vaginal wall, the presence of Döderlein's bacillus and of lactic acid in the vaginal secretion. He has demonstrated that this exists also in higher monkeys but not in other animals and has made experimental observations with regard to the influence of the ovary on vaginal secretion. Observations on women following oophorectomy have also been made which corroborate the experimental work. According to Miura the amount of lactic acid produced in the human vagina in twenty four hours varies from 15 mgm. to 8 mgm. and the hydrogenion concentration runs parallel with the amount of lactic acid. Glycogen is laid down in the superficial layer of epithelium of the vagina, only when the ovaries are present and are actively functioning. Removal of the ovaries or cessation/

cessation of function, e.g., at the menopause, results in absence of glycogen, and alkaline reaction of the vaginal secretion, and a heterogeneous bacterial flora, with absence of Döderlein's bacillus. Hysterectomy does not affect the character of the vaginal fluid. Adler (1928), by means of excisions of vaginal mucosa in the human, has determined the presence of a "functional" layer in the vagina, but the method is of no practical importance. Gisbertz (1929) has pointed out a correlation between the quantity of glycogen and the height of the epithelium. He finds no definite relationship between the glycogen content and the time in the menstrual cycle. He suggests that the intercellular interstices are filled with glycogen and that its presence in the epithelium is responsible for the latter's appearance of "Auflockerung" ("loosening"). Stieve's observations, (1925), of leucocytes in the intercellular spaces have been confirmed.

At this point it is of interest to review our knowledge of the vagina of lower animals/

animals and to compare it with what has been written above. Apart from the cyclic changes associated with the oestrous cycle which occur in some, and which are discussed later in this work, the striking features of the animal vagina are the alkalinity of the secretion, the absence of glycogen and of Döderlein's bacillus. This has been demonstrated in cats, rats, dogs, rabbits, and guinea-pigs (Miura). In experiments on animals, Cahanesco (1901) has found that the self-cleansing power is relatively feeble and differs from animal to animal and from organism to organism. He believes that the direction of the current and the continual epithelial desquamation mechanically carries organisms to the vaginal outlet and that leucocytosis is of importance only after the artificial introduction of large numbers of organisms. Bremicker (1927) states that in the vagina of the pig, lamb, guinea-pig and squirrel the reaction is alkaline and there is no formation of glycogen. In the vaginal wall of these animals small amounts of glucose are found.

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The vagina of squirrels, etc., is self-cleansing, this being assisted in some animals (rat and guinea-pig) by leucocytic infiltration. In the latter animals, the vaginal mucosa consists of squamous epithelium, but in the squirrel the epithelium is of the cylindrical type. In most other species, however, it is of a low cuboidal or flat type. In all animals examined carefully there is a distinct difference from the human, in that the vagina of the latter contains abundant bacteria. In these animals there is an entirely different mechanism which protects the genital canal from the passage of bacteria into the vagina. The alkaline vaginal secretion has no bactericidal action, the self-cleansing power being predominant, and existing in the squirrel, for example, only so long as the cylindrical epithelium is intact. In the mature, long-tailed monkey, however, the vaginal secretion is slightly acid, numerous vaginal bacilli are present and glycogen is found in the vaginal wall. Strains of bacilli isolated from the vagina of the monkey, have been shown by Miura to be indistinguishable from/

from human strains. Man and Monkey are the only animals in which glycogen has been demonstrated in the vaginal epithelium.

Since 1929, two papers of importance on vaginal glycogen have been published: the one by Daniekhy, on "The Importance of Glycogen in the Biology of the Vagina," is in Russian and has not been available to me for study, and the other is by Gisbertz (1930), on the "Morphology of Glycogen Deposits in Epithelium." Reference to the latter work has already been made.

The salient feature of all this work within recent years has been undoubtedly the new conception of the influence of ovarian endocrine activity on the glycogen of the vaginal epithelium, and, secondarily, on the chemistry and bacteriology of the vagina. This is, of course, a matter not only of scientific interest, but cannot fail to be of great clinical importance, which I hope to demonstrate later in this work. The ovario-vaginal inter-relationship indeed merits our full consideration and it is both interesting and instructive to review briefly/

briefly the steps in our knowledge which have led to the conceptions of to-day.

Ovario-vaginal Relationship.

As early as 1889 and 1892, cyclical changes in the vaginal secretion and vaginal wall of rodents were described by Morau and by Lataste. The significance of their findings was practically lost sight of and certainly not appreciated until the work of Stockard and Papanicolaou in 1917. It has since been found that in numerous species of animals, the condition of the vaginal secretion is more or less indicative of the state of the internal reproductive organs. Stockard and Papanicolaou discovered that in the guinea-pig there is a change in the vaginal secretion, recurring just before each ovulation, by which the ovulation cycle can be accurately followed during life. The vaginal fluid contains leucocytes and desquamated epithelial cells. Between the periods of oestrus, cells of both types are present but not very numerous. A few hours before ovulation, however, the leucocytes disappear/

disappear and there is a general desquamation of cornified epithelial cells, which are often shed from the vaginal wall in large sheets. A few hours later, the leucocytes reappear in great numbers and the epithelial cells disintegrate. This vaginal-smear method has proved of the greatest value in following the oestrus cycles of other animals, and has been applied to the mouse (Allen, 1922), to the rat (Long and Evans, 1922), to the opossum (Hartmann, 1923), and to a primate, Macacus Rhesus, (Corner, 1923). The changes in the secretion have been utilised by Allen and Doisy in elaborating the most useful method for recognising and assaying the female sex hormone (Oestrin).

Corner's work is of special interest, concerning as it does an animal not very far removed from the human species. He found a slight tendency towards a rhymical variation in the type of the vaginal secretion. The leucocytes did not suddenly disappear completely in the manner which indicates the time of ovulation in rodents, nor was there the massive desquamation/

desquamation of epithelial cells, followed by leucocytic infiltration, as seen in those animals. There was, however, an increase of epithelial desquamation during the latter half of the inter-menstrual interval and an increase in the glycogen content of the desquamated cells during the same phase. Papanicolaou (1925) claimed a diagnostic value for vaginal spreads in early human pregnancy but no confirmation of his work has been published. All other investigators have failed in their efforts to utilise the vaginal smear in the human. King in 1926 applied the vaginal-smear technique in the examination of apparently normal, healthy women. She came to the conclusion that the cellular content of the normal human female vaginal secretion is exceedingly variable and is a doubtful index of changes transpiring in the ovary. A slight periodicity in the cell content of the secretion is sometimes noted, but this is even less evident than that described by Corner for the monkey.

It must be accepted therefore, from the foregoing/

foregoing remarks, that there is no definite evidence of a periodicity in the cell or bacterial content of the human vaginal secretion, such as could be correlated directly with ovarian cyclic changes and activity. Cyclical and functional changes in the vaginal wall, however, have been demonstrated. In the rabbit, Tsu-Zong-Yung (1924) has reported a vaginal cycle, with cyclic modifications of the mucosa, corresponding to oestrus. Lehmann (1921) has, in the human, described changes in the vaginal bacterial flora which he attributes to cyclical variation of the glycogen content of the vaginal epithelium. He states that in the pre-menstruum and especially during pregnancy, the glycogen is increased and that the glycogen content of the vaginal epithelium runs parallel with the ovarian cycle and is correlated with maturation of the follicle. Gisbertz (1929), however, has been unable to find any correlation between the glycogen content of the epithelium and the time in the menstrual cycle. Stieve (1925) has stated that the vaginal epithelium in humans is definitely thicker and more spongy in the pre-menstruum and has come to the/

the conclusion that the epithelium plays a considerable part in menstrual cycle changes. Dierks (1927), by means of excisions of human vaginal mucosa has also arrived at the same view, namely, that the vaginal mucosa has a strict dependence on the cyclical functional processes of the sexual apparatus. He claims to have produced an increase in the thickness of the vaginal mucosa in a young ovariectomized girl by the administration of "ovarian-active substance," (1929). Adler (1928), by excision of mucosa, has established the occurrence of certain cyclical changes in the epithelial layer, which somewhat resemble the changes found in rodents but to a much less striking degree. Puccioni (1927), Keller (1930) and Gisbertz (1930) also discuss a cycle in the vaginal epithelium in the human correlated with ovarian activity.

Further evidence of ovario-vaginal relationship is furnished by a study of the morphology of menstruation. Geist (1929), in a study of a hundred cases, has found, in menstrual blood, vaginal epithelium throughout in ninety-five to one hundred per cent. of cases.

In/

In shed blood, not connected with the menstrual period, vaginal epithelium is rarely found. This is quite definite evidence of cyclical shedding of this epithelium, specifically correlated with ovarian activity.

It may be of interest to point out that in the condition of intermenstrual pain ("Mittelschmerz"), there is frequently an accompanying leucorrhoea. As far as I am aware, there is no evidence as to whether this discharge is actually vaginal in origin or uterine. But it is conceivable that ovulation, considered by many to be associated with "mittelschmerz," is in these cases accompanied by some disturbance of the vaginal epithelium and excessive secretion from the latter.

In summarising our present knowledge of the ovario-vaginal mechanism, Marshall states that there is in most, if not in all, mammals, a vaginal cycle occurring in association with the uterine cycle. It is to be noted too that the injection of oestrin causes extensive growth in the/

the vagina of the babboon or monkey.

There can be little doubt therefore, of ovarian control and regulation of the vaginal epithelium.

This review then, epitomises our present knowledge of the chemistry of the vagina and more particularly of glycogen in the vaginal mucosa and its relationship to ovarian control and to the biology of the vagina. I have considered it advisable that many of these observations should be confirmed and also extended, and that their clinical significance should be investigated. Accordingly, I have made extensive histological studies of glycogen in the vaginal epithelium from foetal life to post-menopausal years, and my results and conclusions appear in the following pages.

Original Investigations.

Technique. Portions of vaginal wall have been excised both from living adults and from post-mortem cases of foetuses, infants, and children. The material has been fixed in alcohol, embedded in celloidin and sections stained with Best's carmine stain. Duplicate sections have been stained with a 2 per cent. solution of iodine. Both techniques have been used for comparison, as the specificity of the carmine method has been questioned (Lee, 1924). In many cases, triplicate sections have been made, embedded in paraffin and stained with Haemalum and Eosin, to serve as a further control. In several smear preparations the plan of Russell (described by Lee), of spitting upon a control slide has been adopted; glycogen is dissolved by diastase of saliva, the latter is washed off in water and the slide is then stained as for Best's carmine. Comparison of the slides assists in properly identifying glycogen.

Material. Sections of vaginal wall have been examined in the following cases:

Case No.	Name.	Age.
1.	Andrews.	Foetus - about 24 weeks.
2.	McEnery.	Foetus - about 28-30 weeks
3.	Downs.	Still-born.
4.	McKellar.	Still-born.
5.	McPherson.	Still-born.
6.	Carlin.	3½ hours.
7.	McCue.	4 hours.
8.	McCartney.	32 hours.
9.	Henderson.	38 hours.
10.	Lamond.	43 hours.
11.	McDonald.	5 days.
12.	Lunny.	9 days.
13.	McGillvray.	11 days.
14.	Gallagher.	12 days.
15.	Smith.	16 days.
16.	McQuillan.	17 days.
17.	Ritchie.	25 days.
18.	Robertson.	1 month.
19.	Foley.	11 weeks.
20.	McClymont.	3 months.
21.	Guthrie.	3½ months.
22.	McGeoch.	8 months.
23.	Coylan.	8 months.
24.	Gordon.	12 months.
25.	Bryce.	15 months.
26.	Henderson.	19 months.
27.	Bridge.	13 years.
28.	Mitchell.	14 years.
29.	Burton.	18 years.
30.	Nolan.	21 years.
31.	Whitton.	22 years.
32.	Murray.	24 years.
33.	Young.	33 years.
34.	Henderson.	49 years.
35.	Fyfe.	54 years.
36.	Hainey.	59 years.
37.	Miller.	61 years.

Vaginal/

Vaginal smears have also been made in 12 of these cases and have been stained for glycogen.

Results.

(1) In each foetal case, glycogen is abundantly present in the cells of the epithelium, which is many-layered and florid. (Figs. 7, 8).

(2) In the still-born cases and in infants from 3½ hours to 5 days old, the findings are the same. (Figs. 9-13).

(3) In infants, from 9 days to 25 days old, the epithelium becomes progressively thinner and less florid and glycogen diminishes considerably (Fig. 14).

(4) In the cases, of ages between 1 month and 19 months, the epithelium appears very thin and atrophic. Glycogen is extremely scanty or absent. (Fig. 15).

(5) Cases 27-31 are cases of Virginal Leucorrhoea. Glycogen is very abundant and the epithelium is florid. (Fig. 16).

(6) Case 32 is one of a young virgin who had bilateral oophorectomy performed two years previously,/

previously, on account of cystic disease of the ovaries. Glycogen is now absent from the vaginal epithelium.

(7) Case 33 is one of a woman who has never menstruated: there is a complete absence of uterus. Glycogen is, nevertheless, quite abundant and the vaginal epithelium is well-formed.

(8) In the menopausal cases, glycogen is very scanty or absent and the epithelium is thin and atrophic (Fig. 17).

(9) In vaginal smears, glycogen can readily be demonstrated in the desquamated, epithelial cells.

A series of photomicrographs illustrating these results appear in the following pages.

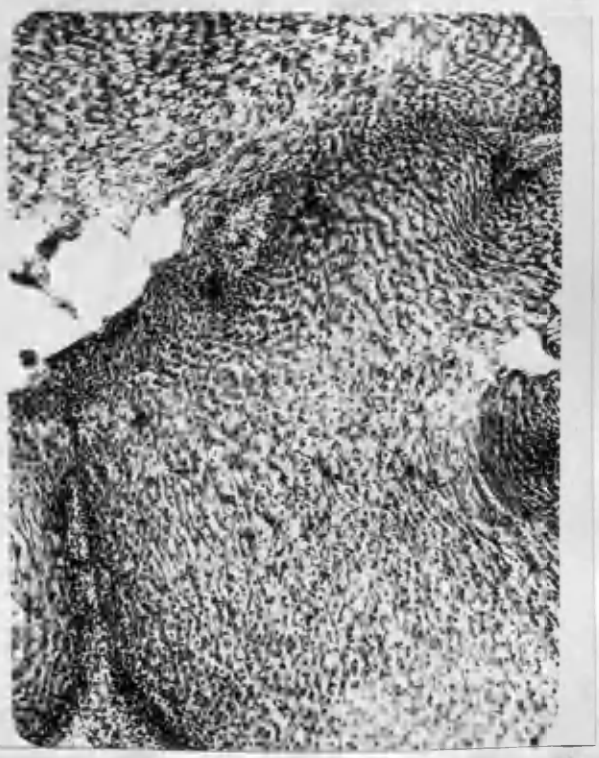

Fig. 7 - Section of vaginal epithelium of six-month foetus, stained for glycogen (x 75).

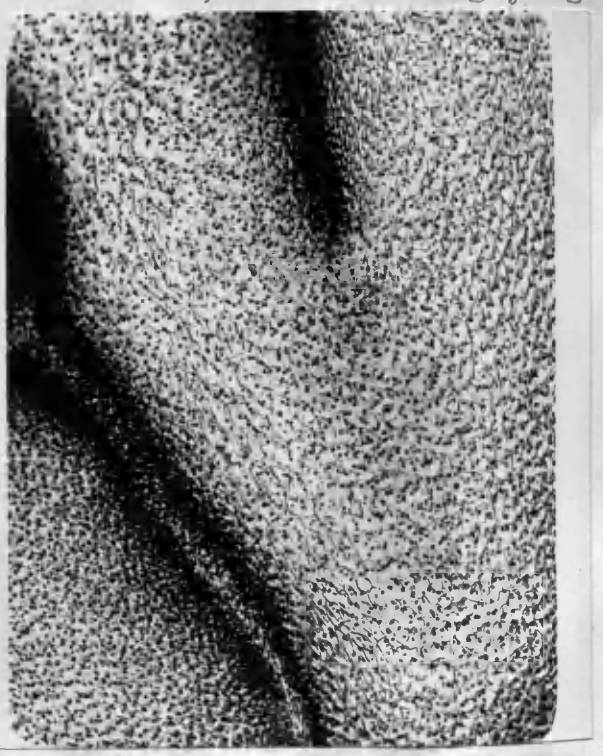

Fig. 8 - Section of same case as Fig. 7, but stained with Haemalum & Eosin (x 75). Note cell spaces, giving "vacuolated" appearance.

Fig. 9 - Section of vaginal epithelium of still-born infant, stained for glycogen (x 100).

Fig. 10 - Same as Fig. 9, but coloured to show appearance, when stained with Best's carmine.

Fig. 11 - Section of vaginal epithelium of six month foetus, stained for glycogen, (x 200).

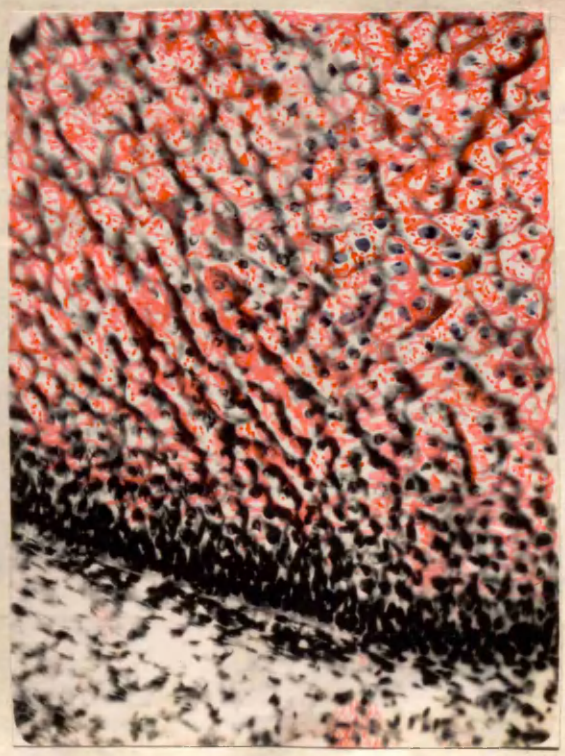

Fig. 12 - Same as Fig. 11, but coloured to show appearance of glycogen, (x 200).

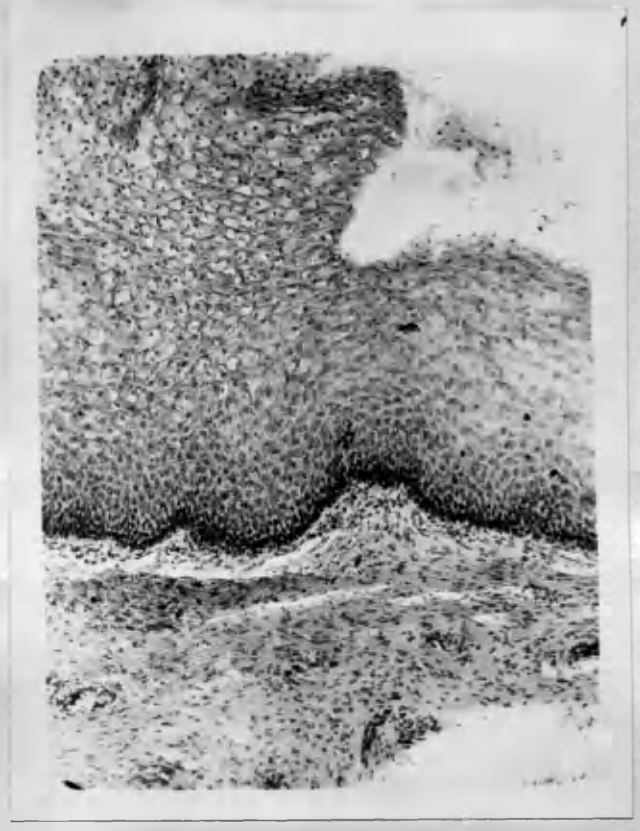

Fig. 13 - Section of vaginal epithelium from infant, aged 5 days -- stained for glycogen (x 100).

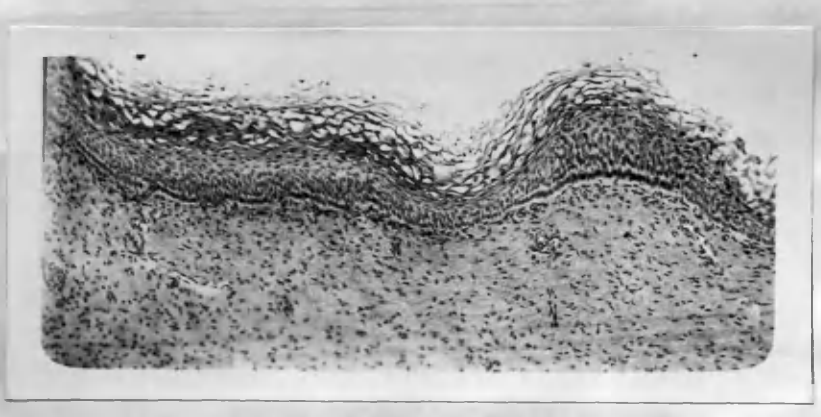

Fig. 14 - Section of vaginal epithelium from infant, aged 25 days -- stained for glycogen (x 100).

Fig. 15 - Section of vaginal epithelium from child aged 15 months (x 75). Note narrow strip of epithelium.

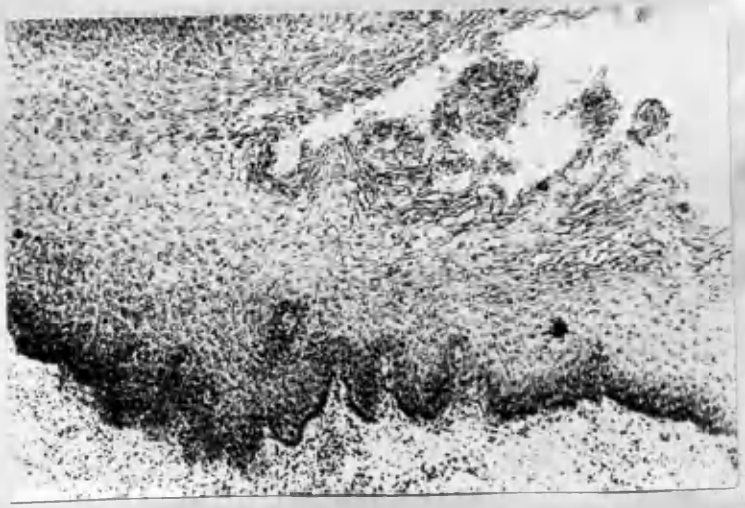

Fig. 16. - Section of vaginal epithelium from case of "virginal leucorrhoea" (x 100).

Fig. 17 - Section of vaginal epithelium from woman aged 54 years (after menopause) - (x 100).

These findings are concisely represented in the following table:

Cases.	Range of Glycogen Content.
Foetus	Very abundant.
Still-born	Very abundant.
3½ hours-5 days ..	Very abundant — abundant.
9-25 days	Small amount — scanty.
1-19 months	Scanty — nil.
13-23 years	Abundant — very abundant.
Menopausal	Scanty — nil.

Observations.

As regards technical results of staining, in this work, there is no doubt that celloidin sections, cut very thin, and stained with Best's carmine gave the most satisfactory results. The nuclei and cytoplasm of the cells stain blue and the glycogen red. The latter appears in the form of intra-cellular granules or small masses, tending to be more abundant at the periphery of each cell (Fig. *10*). Gisbertz (1929) quotes the statement of Romeis that glycogen in living cells is present partly in a diffuse form and partly as granules. He believes that the process of fixation plays a considerable part in giving

a/

a granular appearance (microscopically) to glycogen. Paraffin sections and also paraffin sections fixed with celloidin coating have been found to be unsatisfactory, as most of the glycogen disappears from the cells before staining. The cell interstices, in all sections in which glycogen is present, appear to be filled with this substance, an observation which has also been noted by Gisbertz (1929). Comparison of numerous sections stained by carmine, with duplicate ones stained by Haemalum and Eosin have brought me to the conclusion that the so-called "vacuolation" in the cells of human vaginal epithelium, described in many of the modern text-books, is an artefact due to the appearance produced by the "washing-out" of glycogen in the course of preparation of the usual, routine stained sections.

In the foetus, glycogen in the vaginal epithelium is very abundant. How early it appears, cannot as yet be stated with precision. I have examined one foetal vagina which showed no glycogen, but I have been unable to arrive at the age of the foetus with any feeling of certainty. Further work

on/

on this point is necessary. The fact remains, however, that not long after the vaginal canal is formed, its epithelial lining becomes engorged with glycogen. The physiological significance of this process and its relationship to ovarian activity in the foetus and new-born infant are discussed later in this work (Chapter IV). It is of interest to note that considerable quantities of glycogen are present in the foetal liver and that small amounts may be detected in most foetal tissues, e.g., muscle (Ballantyne, 1902). In all the cases of the series, one fact is strikingly demonstrated in the course of a study of the sections, namely that the height or thickness of the epithelium bears a close relationship to the amount of glycogen which it contains. It would appear as if the epithelium increases in thickness in order that it may contain a greater quantity of glycogen. The close resemblance between the vaginal epithelium of the foetus and the infant during the first few days of life and that of cases of the non-infective type of virginal leucorrhoea, is very striking. No less noteworthy/

noteworthy is the close similarity between the epithelium of the child and that of the woman past the menopause. The sequence of glycogenic activity in the vagina may be expressed thus — it is practically absent from a time soon after birth until puberty, abundantly present from puberty to the menopause and practically absent from the menopause (natural or "operative") onwards. There can be no doubt that this is correlated with ovarian activity. The actual mechanism involved in this relationship and in the changes in the vagina of the foetus and new-born infant requires further consideration.

CHAPTER IV.

THE VAGINAL SECRETION FROM BIRTH TO PUBERTY.

Review of Literature — Method of Estimating Reaction (pH concentration) — Original Investigations — Evidences of Hormone Activity in the New-born — Relationship of Ovarian Histology to Changes in the Vagina.

Review of Literature.

Our knowledge of the characters of the vaginal fluid and of the vaginal flora of children from birth to adult life is not well established. A few accounts by early workers have appeared, concerning comparatively few cases and the conclusions reached have been that the vaginal secretion in young girls is scanty in amount, alkaline and free from Döderlein's bacillus. The more recent work is of conflicting nature, (Cruickshank and Cruickshank). It has long been recognised that at birth the vagina is sterile. Schmidgall (1914) has studied the sterility of the infant vagina and has found bacteria one day after birth/

birth. He considers that the character of the vaginal secretion of the infant is influenced by the vaginal flora of the mother, his explanation being that contamination of the vulva of the new-born occurs in breech presentations. At birth the face and lips of the child cannot but be contaminated by organisms from the maternal vagina. Schweitzer (1919) has recorded the finding of Döderlein's bacillus in the mouths of 46 per cent. of newly-born children, whereas in children delivered by Caesarean Section, he found no evidence of vaginal organisms. He has found the bacillus in the vagina of all cases examined on the third day and supports the view first propounded by Krönig[1] (1894) that it reaches the child from the vagina of the mother. Thomas, (1928) however, believes that the vaginal bacillus in the new-born is derived from the faeces. As Tissier (1900) has found that the first appearance of bacteria in the intestine varies from 10-20 hours after birth, it is somewhat difficult to accept Thomas's view as wholly/

wholly correct. In 12-24 hours, it is generally stated, vaginal smears show the presence of the characteristic Döderlein's bacillus. This is erroneous as I shall show later. One year after birth, Schmidgall states, B. coli communis and Döderlein's bacillus can be demonstrated. Kessler and Rohrs (1927) report that in forty-two children, from 1 to 8 days old, vaginal bacilli were present in all cases (with streptococci in nearly all, staphylococci in many, and B. coli in a few cases) and that the pH varied from 4.0 to 6.0. In thirty-eight girls aged from 9 days to 13 years, vaginal bacilli were found in all cases together with other organisms. They emphasize the importance of examining healthy children, since Wolfring (1922) has shown that the deposition of glycogen in the vaginal wall may be upset by constitutional disease, e.g., tuberculosis. Ulrich (1931) states that at the end of 24 hours after birth, very few Döderlein bacilli appear, but that from the third and fourth days onwards, they become more numerous. Thomas (1928),/

(1928), in the examination of girls between 6 and 12 years of age, found Döderlein's bacillus only twenty times in 107 smears. He noted very few organisms of any kind in smears or cultures. Miura (1928), on the other hand, reports that the vaginal secretion of young girls is alkaline, contains few organisms of any kind and that Döderlein's bacillus is absent until about the twelfth year when a change occurs associated with the onset of puberty; the secretion then becomes acid and Döderlein's bacillus becomes established. Schröder (1930) states that the acidity is greater in the infant and virginal girl than in the adult woman, and that this is so because of the absence of uterine secretion in the vagina of the former. Soeken (1926) describes the sudden change over from a coccal to a bacillary flora, which she observed in a girl just before the onset of the first menstrual flow. From an extended series of observations, involving 500 children of ages varying from 6 months to 9 years, she believes that the change in the vaginal flora is the first definite/

definite indication of puberty and occurs before the secondary sex characteristics develop. In 43 per cent. of her cases, sparse cocci and individual bacilli were found, in 38 per cent. a moderate number of cocci with a few slender bacilli, and in 19 per cent. (mostly children under 2 years) a rich cocccal flora with slender rods in moderate numbers. In a further series of 73 girls, 10 to 15 years old, a coccal flora was found in 39 cases and a bacillary flora in 34 cases. In all of the latter group puberty changes were present, whereas in the former only 7 showed evidence of puberty.

That the reaction of the vaginal secretion of new-born infant is acid is undisputed, owing to the work of Döderlein, Peri, Krönig, Natwig, V. Jaschke, Kienlin and others. Döderlein, however, wrongly ascribed the acidity in the new-born before organisms had yet appeared to carbonic acid and believed that the bacilli produced lactic acid, which was not present in the new-born vagina until organisms appeared. Ulrich (1931) has

obtained/

obtained the following results by introducing small litmus-paper balls into the vagina of the new-born: in 3 cases, aged 1 to 6 hours, reaction feebly but distinctly acid; in 7 cases, aged 5 to 20 days, reaction frankly and rapidly acid.

The presence of glycogen in the vaginal wall in the foetus has been demonstrated by Gragert (1926), and in new-born children by several investigators (Niderehe, 1923; v. Jaschke, 1925; Kienlin, 1926; Kessler and Uhr, 1927). Its association with acidity and Döderlein's bacillus has been pointed out. But it must be remembered at this point that Kienlin has definitely demonstrated, by the specific method of Mendel and Goldscheider, lactic acid in the vagina of the new-born before organisms have yet appeared. He maintains that a fermentative process is then at work on the glycogen of the epithelium. His work supports the view of Loeser (1925), who has estimated the lactic acid content (Warburg's method) of excised vaginal/

vaginal mucosa, kept in Ringer solution. He showed that lactic acid could thus be produced in large quantities. According to Menge (1925), Loeser has also done this in the new-born. The acid reaction persists for three to four weeks after birth, when the vaginal secretion becomes much reduced in amount and alkaline in reaction (Menge).

The precise changes, both in the bacterial flora and in the reaction (pH), of the vaginal secretion from birth to puberty, can thus be seen to be imperfectly known and, accordingly, I decided to investigate this problem and to combine with it a study of the correlated glycogen of the vaginal epithelium. Before detailing the results of this investigation into the bacteriology and chemistry of the vagina in the infant and child, it is essential that some account should be given of the method which has been employed to measure the reaction (pH concentration) of the secretion. All the earlier investigators, e.g. Döderlein, employed litmus paper and were able to assess the reaction merely/

merely as strongly acid, weakly acid, neutral or alkaline. During recent years, an attempt has been made to estimate the reaction more precisely, namely, in terms of Hydrogen-ion concentration (pH). Zwozinski and Truszowski (1926), Schultheiss (1929), Schröder (1930) and Logan (1931) have each made investigations in this direction, employing colorimetric methods. Logan's method consisted of the introduction into the vagina of absorbent papers, impregnated with a mixture of indicators covering a suitable range of pH.

Method of Estimating Reaction.

It was suggested to me by R. Cruickshank that a microcolorimetric method should be employed in my studies of the secretion. On investigation, it was found that in urine examinations the method of Henderson and Palmer had been universally employed for several years after its description in 1912. Since the introduction, however, of the sulfonephthalein series of dyes by Clark and Lubs in 1917, these indicators gradually replaced the earlier ones. The method was modified by Palmer, Salvesen/

Salvesen and Jackson (1920). Fiske (1921) employed the indicators methyl red, brom-cresol purple, phenol red and cresol red. Myers and Booher (1924) suggested a simple technique in which application was made of the dicolorimeter and the phthalein dyes, phenol red, brom-cresol purple and brom-cresol green. Employing these indicators, Ellis designed the capillator (British Drug Houses) for the microcolorimetric method of estimating the reaction of small quantities of fluid. As Cruickshank and Baird (1930) had found this method satisfactory in estimating the pH concentration of vaginal secretion in pregnant women, the former recommended its use in my investigations.

The capillator is analogous to the larger-sized comparator in common use for testing the pH of media, etc. and consists of a series of capillary tubes filled with buffer solutions containing an indicator. These tubes are mounted, three together, on a white card, and form a series of coloured strips, each strip being marked with its exact pH value. Each card illustrates the complete colour/

colour change of the indicator. The whole series of colours being visible all the time, the actual colour matching can be carried out very quickly. The pH is determined by using a capillary tube as a pipette, measuring equal quantities of liquid and indicator and drawing the mixed liquids back into the capillary tube. The colour of the next liquid is then matched against capillator standards (Britton). It is to be noted that, although a colorometric method cannot be expected to be as accurate as the electrometric in estimating pH, the margin of error is very small. Myers and Muntwyler (1928) in a study of 67 urines have assessed the error of the method, and, by employing a correction factor of 0.2, have brought their colorometric estimations within 0.05 pH of the correct values in 80 per cent. of their determinations. Hastings, Sendroy and Robson (1925), also, have controlled their results by the electrometric method and, by subtracting 0.1 pH from the observed colorometric value, have found that their/

their findings agreed within = 0.1 pH of the true value. I, too, have had an electrometric control done in two specimens of fluid, when a very close correspondence in the results was obtained. There can be little doubt that in the case of vaginal secretion, the margin of error in the capillator method is almost negligible.

The method, which I have employed, of obtaining secretion has been that of introducing separately two, long-handled, specially-constructed spoons into the vagina and withdrawing in each some secretion. The reaction (pH) has been estimated by the capillator method. Each spoon has been used for mixing a different indicator, thereby ensuring a check on the colour change in each. In all cases, a fine sterile swab has also been introduced into the vagina, and smears and cultures have been made. The smears have been stained by Gram's method: the cultures have been made on serum agar.

Original Investigations into Vaginal Secretion in Infants and Children.

The material employed has been new-born infants/

infants in the nurseries of the Glasgow Royal Maternity and Women's Hospital, and children in the surgical wards of the Glasgow Royal Hospital for Sick Children. The pH (Hydrogen-ion concentration) of the secretion and the bacteriology of the vagina have been investigated at the following ages: day of birth, second, third, fourth and ninth days, at the end of the third and sixth weeks, and at varying ages from 18 months to 11 years. A detailed account of the cases and of the results is given in Appendix A (infants) and Appendix B (children).

A concise review of my findings is as follows:

A. Reaction of the Infant Vagina:

1. During the first 24 hours of life (53 cases):

pH 5.3 – pH 6.4 in 49 cases;

secretion nil or extremely scanty in 4 cases.

(3 very early cases:- (a) 15 minutes after birth - pH 5.5; (b) 20 minutes after/

after birth - pH 5.7; (c) 30 minutes after birth - pH 5.4).

2. From 24-48 hours after birth (101 cases):

pH 4.9 – 6.4 in all cases.
(pH 5.2 or less in one-third of them).

3. From 48-72 hours after birth (50 cases):

pH 4.7 – pH 5.0 in 32 cases (64 per cent.);
pH above 5.0 in 18 cases.
(In one instance, pH 6.1 and in another pH 6.8).

4. From 72-96 hours after birth (93 cases):

pH 4.8 (average).
(In 5 cases, secretion nil or extremely scanty or red discharge present; in five cases, only, pH exceeded 4.9).

5. Ninth day after birth (90 cases):

pH 4.9 (average);
(Secretion nil or extremely scanty in 9 cases).

6./

6. Three weeks after birth (20 cases) :

In 9, secretion extremely scanty and, for this reason, pH estimated only with great difficulty - pH more than 6.8 (?) ; In 5, secretion nil; in 4, pH 5.0 – 6.0; in 1, pH 6.8; in 1, pH 7.6.

7. Six weeks after birth (30 cases) :

In 11, secretion extremely scanty and, for this reason, pH only estimated with great difficulty - pH over 6.8 (?) ; in 10, secretion nil; in 3, pH 6.0 – pH 7.0; in 2, pH 5.0 – pH 6.0; in 1, pH 7.0 – pH 8.0; in 1, pH 4.9; in 1, pH 4.8.

8. A few miscellaneous cases at varying ages:

(1) 10th day - pH 5.6; (2) 11th day - pH 7.4; (3) 11th day - pH 5.2; (4) 11th day - pH 6.4; (5) 12th day - pH 5.6; (6) 13th day - pH 6.4; (7) 13th day - secretion nil; (8) 19th day - pH 6.8+; (9) 8 weeks - pH 7.6.

B./

B. Bacteriology of the Infant Vagina:

1. During the first 24 hours of life (53 cases):

Smear --- in 51 cases, no organisms;
in 1 case, a few scanty B. Döderlein;
in 1 case, diplococci (23¾ hours after birth).

Culture - in 33 cases, sterile;
in 14 cases, scanty staphylococci;
in 1 case, numerous staphylococci;
in 5 cases, other organisms - scanty.

2. From 24 to 48 hours after birth (99 cases):

Smear --- in 35 cases, no organisms;
in 19 cases, presence of organisms doubtful;
in 14 cases, cocci (mainly enterococci);
in 23 cases, B. Döderlein present;/

present;

in 8 cases, B. Döderlein and enterococci.

Culture - in 1 case only, sterile;

in 6 cases, B. Döderlein;

in the other cases, various organisms, mainly staphylococci, enterococci and B. proteus.

3. From 48 to 72 hours after birth (50 cases):

Smear --- in 30 cases, B. Döderlein abundant;

in 12 cases, B. Döderlein scanty;

in 8 cases, organisms absent or doubtful;

Culture - in 10 cases, B. Döderlein;

in the other cases, mixed organisms.

4. From 72 to 96 hours after birth (99 cases):

Smear --- in 92 cases, B. Döderlein abundant;

in 4 cases, B. Döderlein scanty;

in 2 cases, organisms doubtful;

in 1 case, enterococci only.

Culture/

Culture - in 38 cases, B. Döderlein;
in the other cases, mixed
organisms.

5. Ninth day after birth (90 cases):

Smear --- in 83 cases, B. Döderlein;
in 7 cases, organisms mixed.
Culture - in 31 cases, B. Döderlein;
in the other cases, mixed
organisms - staphylococci,
enterococci, proteus,
pyocyaneus, etc.

6. Three to six weeks after birth (50 cases):

Smear --- in 13 cases, B. Döderlein
predominant;
in 16 cases, B. Döderlein scanty;
in 21 cases, organisms mixed or
scanty or absent;
Culture - in 5 cases only, B. Döderlein;
in 45 cases, various organisms,
mainly B. proteus and staphy-
lococci.

C./

C. Reaction of the Vagina in Children (30 cases):

Ages - 1½ years to 11 years.

Quantity of secretion — small in 3 cases, very scanty in 9, nil in 18.

Reaction:

a. In 18 cases, impossible to estimate pH, owing to absence or extreme scantiness of secretion;

b. In 8 cases, pH 7.4 — 7.8;

c. In 4 cases, pH over 6.8 but exact estimation unreliable owing to insufficiency of secretion.

D. Bacteriology of Vagina in Children (30 cases):

Smear --- in 10 cases, organisms absent or doubtful or scanty and indistinguishable;

in 9 cases, organisms resembling closely B. Döderlein (frequently mixed flora);

in 11 cases, Gram-positive cocci, diplococci and diphtheroids.

Culture/

Culture - in 9 cases, B. Döderlein;
in 21 cases, B. coli, staphylococci, enterococci, streptococci, B. proteus and sporing organisms.

The following tables illustrate at a glance the average, characteristic features at all ages investigated:

Days after birth.	0-1	2	3	4	9
Smear	Cells + Bact. -	Cells + Bact ±	Cells ++ Bact. +	Cells + Bact. ++	Cells + Bact. ++
Culture	Nil	Nil or scanty	Scanty or num. Död. or mixed.	Numerous B. Död.	Numerous Död. or mixed.
Reaction (mean pH) pH range	5.7 5.3-6.4	5.6 4.9-6.4	4.9 4.7-6.1	4.8 4.7-5.6	4.9 4.7-5.6

Days after birth.	21-28	42-56	Childhood
Smear	Cells ± Bact. ±	Cells ± Bact. ±	Cells ± Bact. ±
Culture	Num. mixed; or scanty Död.	Num. mixed; or scanty Död.	Scanty mixed.
Reaction (mean pH) pH range.	6.4 + 5.0-7.6	6.4 + 4.9-7.6	7.6 Secretion nil-pH 7.8

The appearances of typical smears, in infants of varying ages, are shown on the following page:

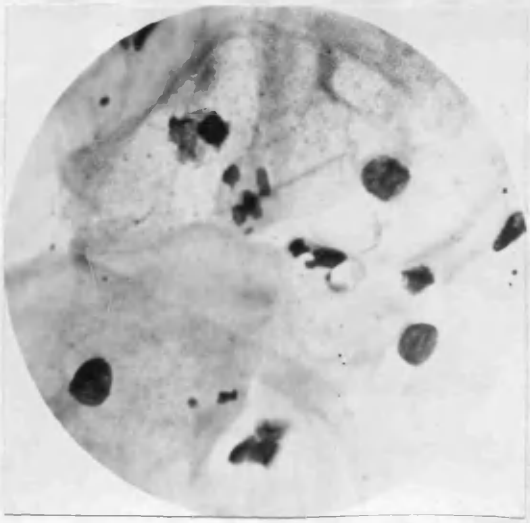

Fig. 18 - Vaginal smear - day of birth. Note epithelial cells: organisms absent. (x 1,000).

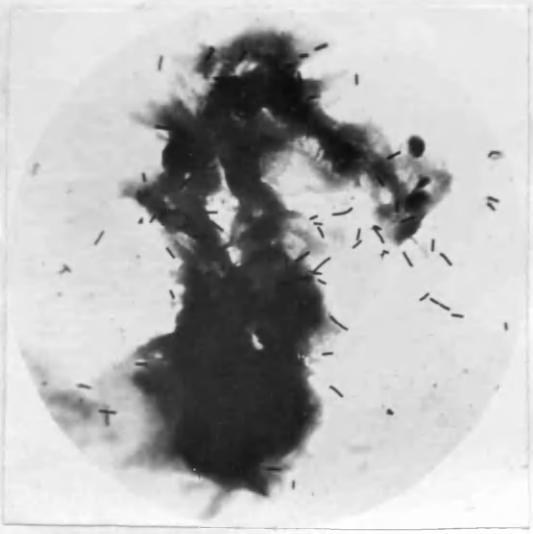

Fig. 19 - Vaginal smear - four days after birth. Note Döderlein bac. and epithelial cells. (x 1,000).

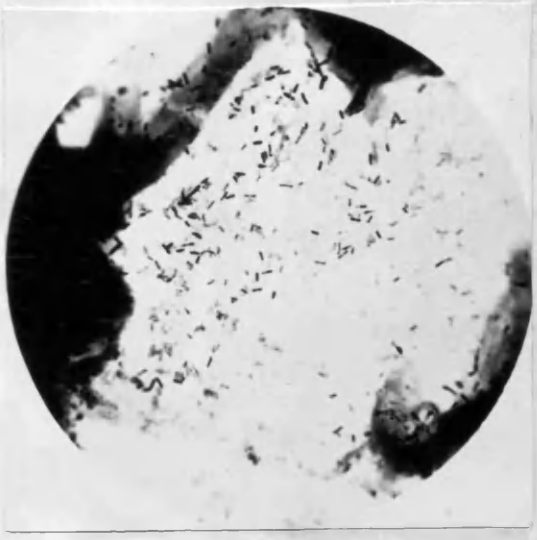

Fig. 20 - Vaginal smear - 9 days after birth. Note scanty cells and mixed organisms, with B. Döderlein. (x 1,000)

Fig. 21 - Vaginal smear - 3 weeks after birth. Note mucoid material and mixed flora; absence of cells and B. Döderlein. (x 1,000)

To summarise the results of my studies in infants and children;

(1) at birth, there is an appreciable amount of vaginal secretion, sterile of organisms, acid in reaction, and containing many vaginal epithelial cells;

(2) on the following day, the secretion tends to increase in amount, a few Döderlein bacilli tend occasionally to appear, and the reaction to become slightly more acid. Many epithelial cells are present:

(3) during the third and fourth days, the secretion becomes excessive in amount and its acidity increases considerably. Döderlein bacilli become abundantly established and epithelial cells appear in greater number: in all respects, the findings at this stage closely resemble those in virginal leucorrhoea. There is little doubt that the striking increase in acidity is due to the action of the bacilli in converting glycogen in the vaginal epithelium into lactic acid. Vaginal haemorrhage/

haemorrhage is occasionally encountered:

(4) by the ninth day, the secretion tends to become more scanty, the reaction to be slightly less acid, the flora to be less homogenous and epithelial cells to be less numerous;

(5) at the third week, in most cases the secretion is nil or extremely scanty (pH not being determinable); when it <u>can</u> be determined, reaction is most commonly weakly acid or alkaline; in the smear, a very mixed heterogenous flora is seen, Döderlein bacilli being present in little more than half of the cases and frequently degenerate in appearance, and epithelial cells are scanty:

(6) the findings at the sixth week correspond closely with those at the third, but more frequently is the secretion nil and the flora heterogenous:

(7) during childhood, in 60 per cent. of cases secretion is nil; when present, its reaction is alkaline; only in 30 per cent. of cases are organisms resembling <u>B. Döderlein</u> present, the flora tending generally to be sparse and mixed; these remain the findings until puberty:

(8)/

(8) the nature of feeding - breast or artificial - does not influence the bacteriological or pH findings, neither does prematurity nor the method of delivery, e.g. Caesarean section:

(9) in two cases of girls, 13 years of age, and in whom menstruation has not yet commenced, I found an abundant Döderlein flora and an ample secretion, highly acid in reaction.

It is obvious then, that from about the tenth day of life, the vaginal secretion in its quantity, reaction, and bacteriology, progressively assumes the features which characterise it from infancy until puberty, when it becomes increased in amount, highly acid in reaction, and shows an almost pure Döderlein flora. The question immediately arises - what is the explanation of the findings during the first few days of life? It seems extraordinary that the vaginal secretion should at this stage present features so closely resembling those occurring as probably the very first signs of puberty and which are typical of the healthy virginal vagina. It seems inconceivable/

inconceivable that they should have a protective or defensive significance as they have in the adult, particularly as the highly acid phase is of such short duration and is not actually fully established until three or four days after birth. But it seems quite probable that the peculiar characters of the secretion in the new-born are of no physiological value and are in a sense accidental. The explanation which one would offer is that the changes are of hormonal origin and are due probably to female sex hormone (oestrin), which has passed over from maternal circulation to foetal and has remained active in the new-born infant for several days after parturition. The vaginal secretion during these days presents features of what one might call a temporary pseudo-puberty. Some support or proof of this theory is called for and it is my opinion that strong support is found in the results of my studies of glycogen in the vaginal epithelium in the foetus, new-born and infant, detailed elsewhere and in the results of my investigations into hormonal disturbance in the new-born, as manifested/

manifested in engorgement of the breasts and in haemorrhagic vaginal discharge, subsequently to be described. I have demonstrated the changes in vaginal epithelium and in the latter's glycogen content from foetal life to childhood, and there can be little doubt of their very close relationship to the nature of the vaginal secretion. It has moreover been quite definitely established elsewhere that ovarian control is exercised over the glycogen of the vaginal epithelium.

One is led to the conclusion, based on these observations on the glycogenic function of the vaginal epithelium and on the characters of the secretion, that the so-called "secretion" is essentially a discharge, consisting of abundant, glycogen-laden, epithelial cells, lactic acid, bacilli and possibly some transudate through the epithelium. I have frequently noticed a large quantity of "discharge" in the vaginal canal of the foetus and I believe that it is similarly produced (although organisms are absent). Ballantyne (1902) believed that it was derived in the foetus from vaginal glands which were physiologically/

physiologically operative, but this is erroneous.

The resemblance between certain phenomena of the first few days of life and those of the onset of puberty is thus very close and common features may be expressed as follows:

(1) Highly acid vaginal secretion, with a pure, Döderlein (Grade A) flora (constant);

(2) Enlargement of the breasts (occasionally in new-born);

(3) Haemorrhage from the vagina, which, as I shall show in the new-born, is occasional and is of uterine origin, is accompanied by endometrial and myometrial changes, and is analogous to menstruation in the adult.

Evidence of Hormone Activity in the New-born.

The precise hormone factor operating to produce the above temporary changes in the new-born, is almost certainly responsible for their more or less permanent appearance at puberty. Frank (1929) states that a large amount of female sex hormone (oestrin) circulates in the maternal blood. It can be recovered from the/

the cord-blood of the new-born foetus (Loewe and Voss, 1926). It therefore follows that the foetuses of both sexes are subjected to its influence throughout intrauterine life unless some special protective mechanism intervenes.

A. Enlargement of the Breasts.

The uterus and breasts of the new-born female child may show activation and are then larger and better developed than those of a child of one year of age (Frank, 1929). The breasts of the new-born male as well as those of the female may show this marked hyperplasia. Halban (1905) ascribed this to a stimulus from substances secreted by the placenta. Since his theoretical explanation, experimental proof of the maternal hormonal stimulus has been adduced by Basch (1909) and others. In a review of 1579 consecutive, new-born infants in the Glasgow Maternity Hospital, I have found engorgement of the breasts present in 76, being an incidence of 4.8 per cent. In females, the incidence is 6.4 per cent. and in males 3.3 per cent, i.e. the/

the condition is almost twice as common in female infants. It may occur in cases where no breast feeding has been employed, but is much more common in breast-fed infants (87.5 per cent.) A striking feature is the high incidence of engorgement of the breasts in cases of neonatal vaginal haemorrhage, engorgement being present in 18 out of 51 cases (35.3 per cent.) of the latter condition. The association between hormonal and mammary activity has, of course, been long recognised. In recent years, evidence has accumulated to show that anterior pituitary lobe can undoubtedly influence and even induce mammary activity under experimental conditions. Whether it can do so independently or only in the presence of ovarian tissue, is still a moot point (Krestin, 1932). Frei and Grüter (1929) have produced such activity, accompanied by lactation in virgin mammals by the injection of anterior pituitary extract.

B. Haemorrhagic Vaginal Discharge.

In the course of the investigations, which I have/

have described, into the bacteriology and reaction of the vaginal secretion in the newborn, cases have been encountered not infrequently, in which red discharge from the vagina made its appearance. It has not been possible in these instances to estimate the pH of the secretion by the capillator, as the red colour completely distorts the colour readings. But I have examined fresh drops, smears and cultures in a number of cases and have been sufficiently fortunate in having been able to remove the uterus and vagina post-mortem, during the existence of red vaginal discharge in an eight-day old infant who died of a congenital cardiac lesion. Owing to an error in technique, I did not remove the ovaries, but sections of the uterus and vagina have been made and examined. Although of no clinical importance, the condition is one of much scientific interest and I shall describe briefly the results of my investigations and observations. In a consecutive series of 993 female infants in the Nurseries of the Glasgow Maternity Hospital, haemorrhagic/

haemorrhagic vaginal discharge has been encountered in 51 cases, i.e. 5.1 per cent., (cf. series of Zacharias, (1914), incidence 2.5 per cent). These cases have been studied and some of their features presented in the subjoined table:

Case No.	Vaginal Haemorrhage.		Engorged Breasts
	Age in days at appearance.	Duration in days	
1.	4	3	-
2.	2	2	-
3.	8	2	-
4.	6	4	-
5.	5	3	-
6.	4	4	-
7.	8	2	-
8.	4	2	-
9.	4	5	-
10.	5	2	-
11.	5	6	-
12.	5	4	-
13.	7	1	-
14.	3	5	+
15.	4	7	+
16./			

Case No.	Vaginal Haemorrhage.		Engorged Breast.
	Age in days at appearance.	Duration in days.	
16.	4	4	-
17.	4	4	-
18.	3	8	+
19.	4	6	-
20.	4	2	+
21.	4	7	+
22.	5	1	-
23.	7	2	-
24.	7	1	+
25.	4	1	+
26.	5	2	+
27.	5	3	-
28.	5	2	-
29.	6	2	-
30.	4	1	-
31.	5	2	-
32.	7	2	-
33.	6	1	-
34.	5	1	+
35.	5	3	+
36./			

Case No.	Vaginal Haemorrhage.		Engorged Breasts
	Age in days at appearance.	Duration in days	
36.	9	1	-
37.	6	2	+
38.	6	1	+
39.	6	2	-
40.	6	2	-
41.	6	2	+
42.	6	3	+
43.	5	2	-
44.	3	4	+
45.	7	1	-
46.	4	5	+
47.	5	1	-
48.	4	5	-
49.	5	3	+
50.	4	5	-
51.	9	1	+

All the infants of this series were wholly or partially breast-fed with the exceptions of/

of cases 2, 48 and 50 which received no breast milk whatsoever. These three cases afford evidence that the conveyance of the maternal hormone to the infant is not wholly via the milk, although it is not disputed that this substance may act as an additional vehicle. The observations shown in the table may be summarised as follows:

1. Vaginal haemorrhage in the new-born most frequently appears on the fourth and fifth days after birth (57 per cent. of cases), less frequently on the sixth and seventh days (27 per cent.) and occasionally on second, third, eighth and ninth. Its onset is not encountered on the day of birth or after nine days.
2. Its duration is in most cases 1-2 days (57 per cent.). It is present for 3, 4 or 5 days in 33.3 per cent., and never for more than 8 days.
3. It has not been seen subsequent to 11 days after birth.

4./

4. Engorgement of the breasts is associated with the condition in 35.3 per cent. of cases. This association is very striking, contrasting with the general incidence of engorged breasts in female new-born of 6.4 per cent., (48 in a series of 748 infants).

Fresh drop and stained-smear preparations of the haemorrhagic discharge in several cases have shown it to possess, in its epithelial elements, characters similar to those of menstrual blood. According to Geist, (1929), the morphology of normal menstrual blood in the adult is constant and sufficiently specific in its epithelial and leucocytic constituents to warrant its differentiation with certainty from blood of other types of genital bleedings. However, my own findings do not justify the making of an equally definite assertion in the case of the new-born. Smears and cultures have shown that the vaginal flora in these cases tends to be more heterogeneous and B. Döderlein more scanty or absent than in infants of corresponding ages but having no vaginal haemorrhage.

The remaining line of investigation in this subject has been the study of the uterus and vagina of an infant who died from cardiac disease during the course of vaginal haemorrhage. To serve as a control, I removed the uterus and vagina, post-mortem, from a "normal" infant of the same age (8 days). Macroscopically, the two uteri show very striking differences;

(1) the "abnormal" one is approximately four times as large as the other in all dimensions;

(2) it shows, on transverse section, a circular zone of congestion or haemorrhage in two sites (a) the lining of the uterine cavity and (b) the myometrium close to the peritoneal surface.

The microscopic appearances of sections from the uteri, stained Haematoxylin and Eosin, are shown on the following page:

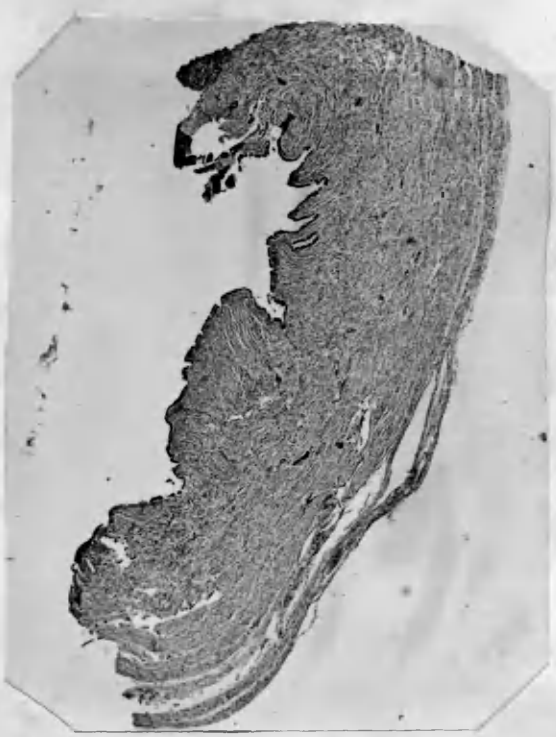

Fig. 22 - Section of normal uterus from 8-day infant. (x 28).

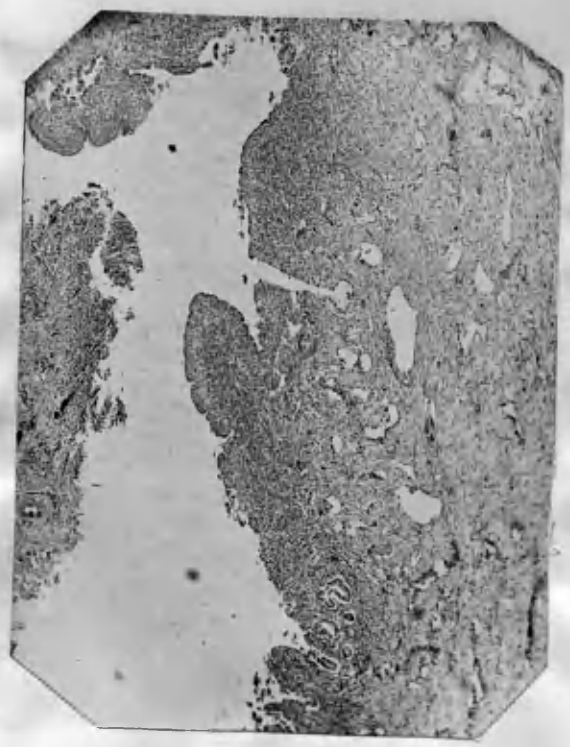

Fig. 23 - Section of uterus from case of "vaginal haemorrhage" in 8-day infant (x 28). Note great thickening of endometrium and myometrium.

The noteworthy features in the "abnormal" uterus are:

(1) Considerable hyperplasia of endometrium and myometrium;

(2) Extensive new formation of vascular channels in myometrium;

(3) Sub-endometrial haemorrhages.

Sections of vaginal wall in this case and in the control one were stained with Best's carmine stain for glycogen. Very little appreciable difference is noted either in the thickness of epithelium or in the glycogen content, but no precise conclusion is to be drawn on these points, in the absence of corroborative material.

It is of interest to note that Frank (1929) has stated that the excitation exerted upon the uterus of the new-born by the maternal female sex hormone regularly produces hyperplasia of muscle and endometrial lining. Zappert (1903) has observed that in certain instances the stimulus may reach such a degree that after separation from the mother, an abrupt retrograde catabolic involution occurs, which is strictly comparable to menstruation of the adult.

There can be little doubt therefore, from these various observations, that haemorrhagic vaginal discharge in the new-born arises from the uterine endometrium, is due to a hormonal disturbance and is analogous to menstruation. It must not, however, be confounded with genital haemorrhages occurring coincidently with haemorrhages elsewhere in the body of the new-born, as the result of sepsis or a general haemorrhagic disease.

Relationship of Ovarian Histology to Changes in the Vagina.

In order to determine if the ovaries of the foetus, new-born and infant show histological differences, more particularly in follicular development, which may be correlated with the changes which I have described in the vaginal epithelium and in the secretion, I decided to study a series of cases. Frank (1929) has emphasised the fact that from the early weeks of life onwards until the approach of puberty, although the female child in many ways gives the picture/

picture of an asexual or neuter state, evidence is at hand to show that a continuous though not large secretion of hormonal substance from the gonad occurs. Anatomical evidence is furnished by the slowly increasing number of growing primordial follicles, which reach early Graafian size and development but then undergo atresia (Hartmann, 1926). This author has described, in the ovary before birth, the development of follicles and the presence of small corpora albicantia. The atretic follicles, according to Frank, by virtue of their proliferating granulosa layer, are able to secrete small quantities of hormone sufficient to control tropic growth, but, quantitatively, follicle ripening is still insufficient to initiate puberty (Frank, 1922). Preliminary experiments show no greater amount of hormone in foetal ovaries than in other foetal tissues.

I have removed both ovaries (post-mortem), in a series of 11 cases of varying ages. In a study involving the recognition and description of follicles and, possibly, of phases of their growth, it is quite obvious that only serial sections/

sections of the entire ovary can be of value and accordingly in all cases I have made a complete series of serial sections. Every twentieth section has been examined (stained – Haematoxylin and Eosin). The following is a list of the cases in which the ovaries have been serially sectioned; (in each case, a portion of vagina has been sectioned and stained for glycogen in the epithelium):

Case No.	Name.	Age.
1.	McEnery.	Foetus - 7 months.
2.	McCue.	4 hours.
3.	Henderson.	38 hours (but premature).
4.	Gallagher.	12 days.
5.	Smith.	16 days.
6.	Robertson.	1 month.
7.	McClymont.	3 months.
8.	McGeough.	8 months.
9.	Coylan.	8 months.
10.	Gordon.	1 year.
11.	Bryce.	1 year, 3 months.

Examination/

Examination of the sections reveals information of interest. The histological appearances, with particular reference to follicular development, will now be briefly described:

Case 1. Foetus, 7 months. The structure of the ovary is identical throughout. Innumerable, small, undeveloped, primordial follicles are seen.

Case 2. Aged 4 hours. Follicular development is present, but only in a few follicles. A considerable portion of the ovary shows no maturation of follicles.

Case 3. Age 38 hours. The structure closely resembles that of Case 1. No evidence of developing follicles is apparent. It is to be noted that the infant was premature.

In all the other cases, abundant evidence of growth of follicles, some closely resembling Graafian follicles of the adult, is seen. Corpora albicantia are encountered frequently. The fallacy of examining merely single sections of an ovary is emphasised, as, in/

in several of the cases, some of the sections showed quite different appearances from others, of the same ovary. The following photomicrographs illustrate characteristic appearances in several of the cases.

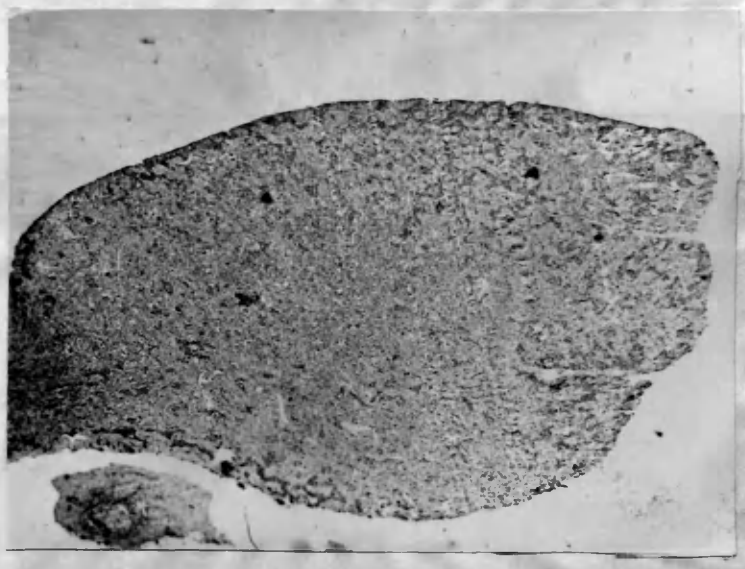

Fig. 24 - Section of ovary of 7-month foetus (x 20).

Fig. 25. - Section of ovary of infant, aged 4 hours. (x 20).

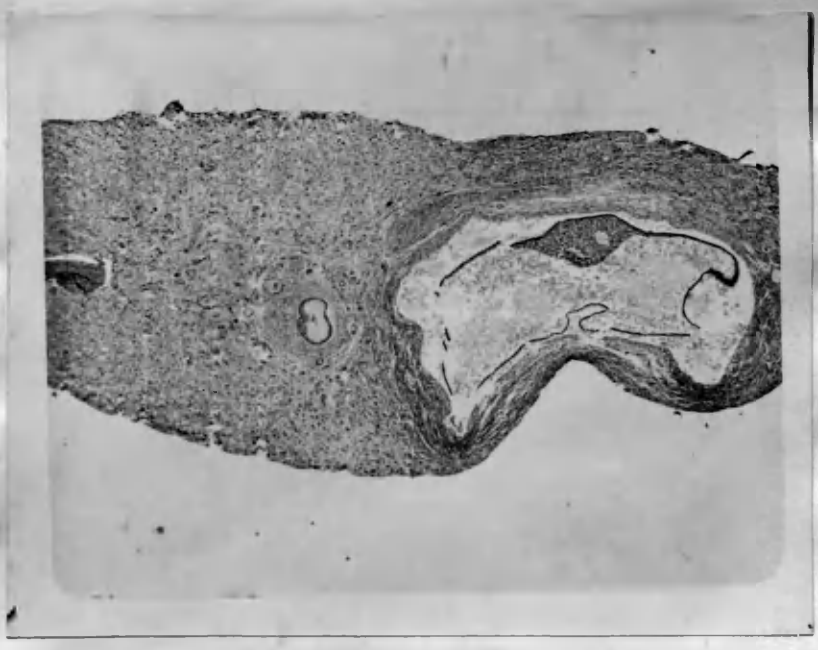

Fig. 26. - Section of ovary of infant, aged 12 days (x 20).

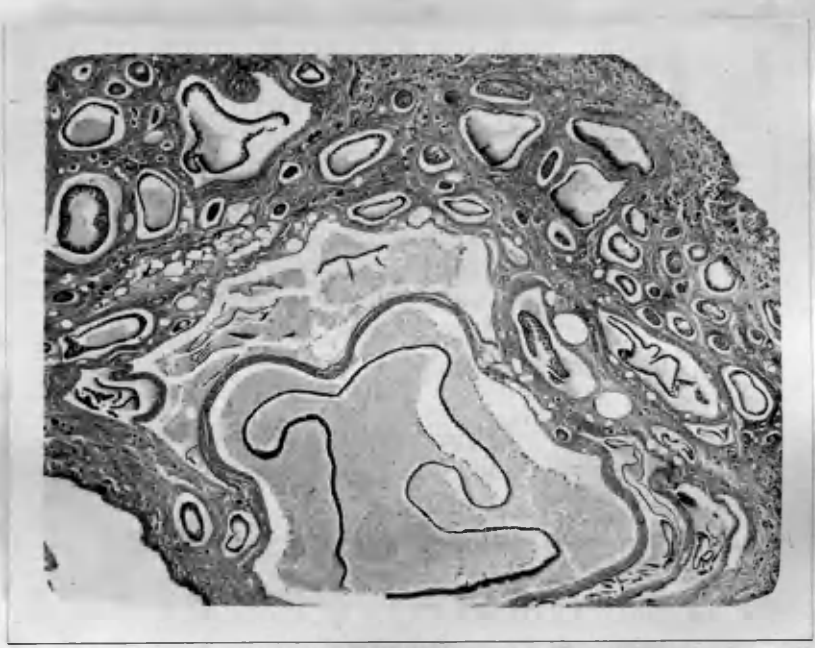

Fig. 27 - Section of ovary of infant, aged 4 weeks (x 20).

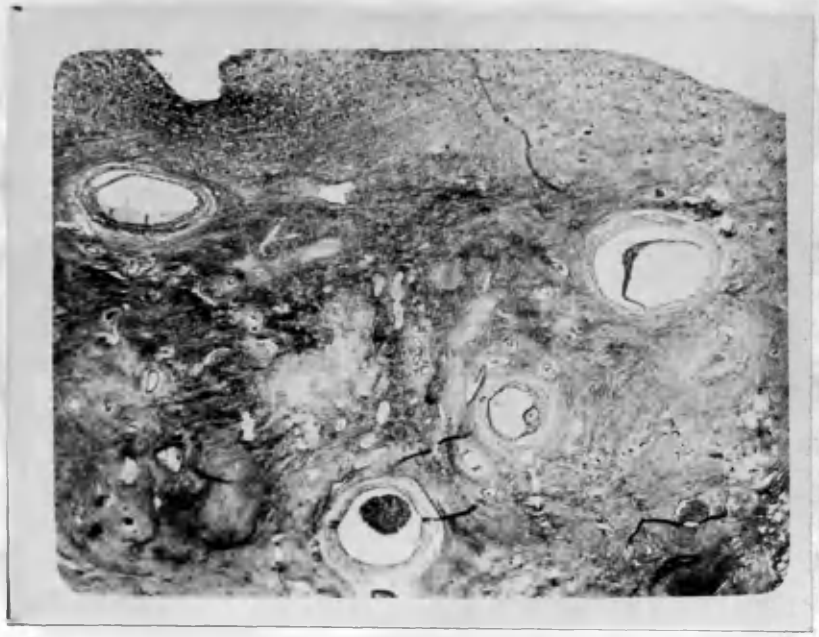

Fig. 28 - Section of ovary of infant, aged 8 months (x 20).

After a careful study of the histology of the ovarian follicles, briefly described above, I have come to the conclusion that there is no evidence in the ovaries of any features which may be correlated with the changes in the vaginal epithelium and secretion of the foetus and newborn infant. On the evidence available, therefore, one arrives at the opinion that the hormonal stimulus responsible for these changes is directly exercised from the maternal circulation.

CHAPTER V.

VIRGINAL LEUCORRHOEA.

The Problem of Pathogenesis — The Gonorrhoea Question — Pathological Causes — Original Investigations — Observations on Etiology — Trichomonas Infection of the Vagina — Fungus Infections of the Vagina — Treatment.

The Problem of Pathogenesis.

In the clinical study of virginal leucorrhoea, I have concerned myself only with cases which showed on examination an apparently intact hymen and in which sex intercourse was emphatically denied. Nevertheless, it is appreciated that we can be only reasonably certain of virginity. Absence of the hymen does not necessarily indicate loss of virginity, neither does its persistence unequivocally point to the existence of virginity. A cribriform hymen with minute openings is almost certain evidence, but semilunar or crescentic hymens, if elastic, may permit of penetration without tearing. Cases, indeed, have/

have been described of pregnancy in women in whom the hymen was apparently intact (Schilling, 1925) and it is not impossible for a gonorrhoeal infection to be present with no local signs of defloration. It is obvious, therefore, that a primary difficulty is here encountered in investigating these cases. However, great care has been taken in reducing the possible margin of error to a minimum. I have regarded "leucorrhoea" as that degree of discharge (other than blood-stained), sufficient to soil the clothes or necessitate the use of a sanitary napkin and regarded by the patient as an appreciable departure from her normal state. The very mild degrees of excessive discharge, pre-menstrually or temporarily during conditions of depressed general health, are of little clinical moment. An excessive discharge from the vagina may reasonably arise from any one of three sources - vagina, cervix and uterus, and one must therefore determine which source is responsible for the leucorrhoea and if possible its cause or causes.

In/

In order to appreciate fully the pathogenesis of excessive discharge from the female genital tract, it is essential that the histology of the tract (more particularly of the "lining membranes") should be clearly understood. The two Müllerian ducts of the embryo, lined by columnar epithelium, fuse to form the Fallopian tubes, uterus, cervix and vagina of the adult. The epithelium becomes converted in the tubes to ciliated columnar epithelium, in the body of the uterus to low columnar epithelium, in the cervix to a non-ciliated high columnar epithelium, with regular basal nuclei, and in the vagina to squamous epithelium. Actually, the change in vaginal epithelium is not due to a conversion of the original columnar epithelium but to a replacement of it by squamous epithelium growing from below, upwards, from the region of the uro-genital membrane. (The peculiar glycogenic character of the vaginalepithelium, as described earlier, seems to be all the more remarkable). Simple tubular glands

appear/

appear in the uterine endometrium and compound or racemose glands in the mucus-secreting cervical epithelium. The vaginal mucosa has already been fully described, but, in brief, to recapitulate, it may be said that it contains no glands and is lined by squamous epithelium. Squamous epithelium also covers the vaginal aspect of the cervix, the canal of which is lined by columnar epithelium. In the six-month foetus, squamous epithelium is found in the cervical canal, but it is later replaced by the columnar type. Fischel (1897) has shown that in thirty-six per cent. of the new-born, the portio is covered with a single layer of cylindrical epithelium, with frequent persistence of cervical glands (congenital erosion). In later life, if the replacing squamous epithelium desquamates, the epithelium from persistent foetal glands covers the denuded surface with a cylindrical layer.

Uterus, cervix and vagina have each their own peculiar, characteristic, normal secretion. It has for many years been recognised/

recognised that the tubular glands of the endometrium are the source of a small quantity of secretion in the normal uterus. Fritsch in 1885 had mentioned this, but had made the mistake of assuming that the normal vaginal secretion mainly consisted of secretion from uterus and Bartholin's gland. Whitehouse (1928) has mentioned the rare condition of "white menstruation," in which a white discharge, consisting of the secretion of the uterine glands occurs, but no haemorrhage from the uterus, although the sexual organs present no apparent anatomical abnormality. My own studies have brought me to the conclusion that a negligible amount of the normal secretion in the vagina, e.g., in a healthy virgin, is of uterine origin. The secretion from the racemose glands of the cervix is thick, mucoid, viscous and alkaline and forms the typical cervical secretion. The vaginal secretion has already been described in detail and its thick, white, cheesy, highly-acid character noted. In the quiescent/

quiescent period of the menstrual cycle, if conditions are normal, these natural secretions are hardly appreciated.

The bacteriology of the normal, healthy, female genital tract is well established. The normal uterus contains no bacteria, the vaginal flora being arrested at the external os (Veit). According to Bumm (1895), Menge (1897) and others, the cervical canal rids itself of introduced pathogenic organisms within a few hours, unless injury to the epithelial covering has occurred, or discharges, clot, etc. offer a favourable habitat. The same applies to the uterine cavity. Exceptions to this rule are organisms which can flourish on an intact membrane, e.g., the gonococcus and diphtheria bacilli. The bacteriology of the vagina has already been described in detail.

Leucorrhoea is, of course, a common complaint. Historically, one finds reference to it in the very oldest of medical literature. Brooke Bland (1931) mentions in this connection Eber's/

Eber's Papyrus, written some fifteen hundred years before Christ. He also remarks that reference is made to the symptom in the earliest biblical literature, which antedates the Egyptian document by some thirty five centuries. It is most common in married women. Davis[1] (1929), has found, in a review of one thousand histories of gynaecological and obstetrical patients, that about 33 per cent. had some type of leucorrhoea. The cause of the symptom in these cases is usually obvious, in most instances being due to cervical infection, which owes its origin generally to childbirth, or abortion or gonorrhoea. Less commonly, vaginitis, due often to Trichomonas vaginalis, is responsible. In virgins, however, the complaint of leucorrhoea is uncommon; it is impossible for obvious reasons, to determine accurately its incidence. Leucorrhoea in virgins has always been a clinical problem which has been difficult/

difficult to explain satisfactorily and which has given clinicians considerable trouble in its treatment. Most gynaecologists have repeatedly encountered such cases and have not only had difficulty in understanding the pathogenesis of the condition, but have also had frequent failures from their therapeutic efforts.

It has, of course, been generally appreciated that constitutional disorders may be responsible for excessive discharge in virgins. This is believed by many to arise as a result of a "low-grade infection of the cervix." Pelvic congestion and constipation have also been blamed as etiological factors. Schauffler (1927), in an article on vaginal discharge in girls before puberty, has pointed out that a simple catarrhal vaginitis is common in high-strung, neurasthenic girls and that often such a discharge is noted only when the child suffers from fatigue or nervous depression. Anaemia, malnutrition, or debility/

debility of any sort are strikingly associated with relapses or exacerbations of the chronic condition. There is an absence of trauma and of repeated exposure to infection. He comments on the really striking feature in the examination of miniature vaginas being the rugose, contracted condition, a state not seen in the non-virginal, because of repeated dilatation from coitus or childbirth. An unruptured hymen serves, further, to obstruct these rugose, contracted vaginas, and all factors are present to constitute a true "harbour of infection:" indeed, in adult virginal women and in old women whose vaginas show the contraction and stasis of senile atrophy, a primary vaginitis is often found. Payne (1929) writes that infection of the nuliparous cervix, the canal of which is normally filled with a thick tenacious mucus, acting as a barrier to the usually sterile canal from contamination from the vagina, generally begins as an endocervicitis and is usually of an ascending type. For this reason/

reason, the brunt of the infection is usually borne by the lower portion of the cervical canal. Burns (1922), also, asserts that in the case of the young girl with intact hymen, infection of the cervix arises by direct spread upwards from the external genitals. This mode of infection, he states, may be denied by those who believe that the vaginal secretion is capable of preventing infection from travelling upwards. In cases of endocervicitis investigated by him, five are stated to have shown certain stigmata usually associated with masturbation. He believes that sexual excitation is accompanied by congestion of the pelvic organs, and, if frequently indulged in, brings about a condition approaching chronic congestion. This congestion induces hypersecretion of the cervical glands, in other words, leucorrhoea. The external genitals become bathed in this vaginal discharge, the bacteria which are always present spread up to the cervix and the glands become infected. Once the glands are infected, the presence of the/

the organisms and their toxins maintain the hyper-secretion and so the condition of endocervicitis is established. Kidd and Simpson (1929) state that under conditions of sexual excitement an almost instantaneous pouring out of secretion occurs from the glands of the vulva and cervix for purposes of lubrication. In modern life this excess of secretion is continually being brought into play by the stimulation of erotic dancing or cuddling, which should in a state of nature end in an act leading to a natural deturgescence of all the glands and the return of the secretion to the normal. These authors state that there can be little doubt that the leucorrhoea of young girls may be brought about by this unnatural stimulation of the parts without natural gratification. Whether this statement is true or not it is difficult to prove or disprove, but for reasons which will be adduced later, I am inclined to attach minimal importance to it.

In an endeavour to assess the results of treatment in cases of leucorrhoea in unmarried/

unmarried nulliparae, in whom, from the data available, there was no evidence of gonorrhoea, I "followed-up" the results of treatment in 76 cases, which had been in the Royal Samaritan Hospital for Women, Glasgow, in the course of a period of five years (1924-1929). There is no certainty that all the women were definitely virginal. The results, however, are interesting and are as follows:

Result of Operation.	No. of Cases.	Percentage.
Not improved	38	50.0
Slightly improved ..	5	6.6
Much improved	14	18.4
Cured	19	25.0

It is apparent that only 50 per cent. of patients benefitted by their stay in hospital. In 23 of the 76 cases, no abnormality in the genital tract was detected and of these 13 were not improved as a result of their hospital treatment. Treatment varied, but

most/

most of the cases had a dilatation and curettage performed, while some had cauterisation of the cervix in addition or alone. In some cases, systematic painting and douching of the vagina with an antiseptic was employed, while in others examination under anaesthesia was the only interference, as so frequently the source of the discharge was not apparent.

It is quite obvious that the knowledge of virginal leucorrhoea is unsatisfactory and that not only does its mechanism require elucidation but its treatment requires to be rationalised and put on a scientific basis. Before detailing the results of study and investigation over a period of three years of some 40 cases, - cases which do not by any means illustrate all the possible causes of excessive discharge - it is advisable that certain definite pathological conditions which may give rise to leucorrhoea in virgins should first be described.

The/

The Gonorrhoea Question.

Firstly, it is desirable to consider briefly the question of gonorrhoea. It is conceivable that a gonorrhoeal vulvitis in the adult may be present without there having been sexual relations, but this accidental infection must be exceedingly rare. Again, in the presence of an intact hymen, it may be due to incomplete relations. In these cases, diagnosis may be difficult, particularly so as the patient may firmly deny intercourse, as she may well do. Lees (1932) states that patients with vaginal discharge fall into three groups: those in whom the diagnosis, clinically and bacteriologically, is gonorrhoea; those in whom the clinical picture and the history suggest gonorrhoea, but who give negative laboratory tests; and those in whom there is no evidence of infection by the gonococcus, but in whom there is evidence of infection by other organisms. The finding of a Gram-negative diplococcus, indistinguishable from the gonococcus, is, of course, most important. Several observers, however,/

however, have found that Gram-negative cocci other than the gonococcus are sometimes found in the female genital tract. Cuizza (1930) alludes to the diagnostic errors, of medico-legal and clinical importance, which may result from confusion of such organisms with that of Neisser. Examining the cervico-vaginal secretion in 65 gynaecological patients, of whom 51 suffered from acute or chronic cervico-vaginal or pelvic inflammation, he found Gram-negative cocci differing from the gonococcus in 6; cultural growth was necessary to establish definitely the fact that these were not gonococci. Clinically, it is impossible to distinguish between a vaginal discharge due to the gonococcus and a discharge due to other organisms (Anwyl-Davies, 1932). Indeed Harrison (1932) states that a large number of vaginal discharges are wrongly diagnosed as gonococcal in origin. Turning to the laboratory again, we find a useful diagnostic weapon in the complement-fixation test. Izwojnicka and Zawodzinski (1931) report

the/

the results of a comprehensive investigation of this test. In a series of 1,495 tests, these authors found that during the first three weeks after infection the reaction was negative in half the number of cases, but after the twenty-first day, the results were almost uniformly positive (94.6 per cent.). In a control series of 831 cases, in which gonorrhoea was not suspected, the results were negative in 97.6 per cent. These investigators conclude that the deviation of complement in gonorrhoea is specific and that a positive reaction is practically certain evidence that a patient has or has had the disease, (or has had a dose of gonococcal vaccine during the previous three months). The value of the complement-fixation test in this field is emphasised by almost all modern authorities. Therefore, one must conclude that recourse to it should be had in those cases of doubtful gonorrhoea in virgins, with which at present we are concerned.

It has been stated (Kelly) that the

reaction/

reaction of the discharge should be tested, as being of value in helping to settle the question of a gonococcal infection. Danin (1925) declares that an acid intermenstrual reaction excludes gonorrhoea; Kelly states that this has been confirmed by Leo Brady in 49 out of 50 examinations, but needs further investigation and confirmation. With this latter object in view, I have investigated (by a method to be described later) the reaction (pH or Hydrogen-ion concentration) in cases of bacteriologically-confirmed gonorrhoea, involving children and unmarried nulliparae only. The results are summarised as follows:

1. In 10 adult cases, reaction of vaginal discharge from pH 5.4 to pH 7.0: in the same cases, reaction of the actual cervical discharge between pH 6.0 and pH 8.4.
2. In every case cervical pH distinctly higher than the vaginal pH (i.e. more alkaline).
3. Even after several weeks intensive treatment, cervical pH almost constant; in the same cases, as infection becomes less acute, vaginal pH tends to fall (becoming more acid), but not below/

below pH 5.4. These are the findings in three cases, even when clinically cured.

4. The following details of one case are illustrative: first examination — vaginal pH 5.7; five weeks later, pH 5.7; seven weeks later pH 5.7; four weeks later, pH 5.6; seventeen weeks later (clinically cured) pH 5.6.

5. In five cases of gonorrhoea in female children, ages varying between 3 and 11 years, the pH values of the discharges are as follows: 7.4, 7.6, 7.4, 6.8, 7.0. These figures differ very little from those of the normal pH (when obtainable) in the healthy vagina of children, as I have shown elsewhere in this work.

Although these investigations were done on a very small series of cases, they were sufficient to indicate that I was unable to confirm the findings of Danin and Brady. True neutrality is represented by pH 7.0, high acidity by a pH below 5.0 and high alkalinity by a pH above 8.0. Accordingly, I have obtained acid intermenstrual/

intermenstrual reactions in gonorrhoea. But I am of opinion that a highly acid reaction of vaginal discharge, such as is found in the case of the normal virginal vagina (pH 4.0 to pH 4.8), excludes gonorrhoea. Therefore, I would stress the value of estimating the reaction of vaginal discharge in all cases of doubt, particularly in virgins. Estimation is simple (by the capillator method to be described later) and its result is a valuable diagnostic adjunct.

Pathological Causes.

We must now consider certain pathological conditions which may be responsible for leucorrhoea in virgins. Although, strictly speaking, a discharge arising from the vulva, does not come under the category of vaginal discharge, yet it may be so described by the patient and may cause some difficulty to the clinician in its differentiation. Infectious diseases such as typhoid, smallpox, scarlet fever, dysentery, diphtheria and measles often show a complicating vulvitis or vaginitis or both. Vulvitis varying in severity from/

from the catarrhal to the gangrenous type may occur. Vaginitis of all degrees of severity in the following fevers has been recorded: typhoid (Keen), dysentery (Eppinger), pneumonia (Bröse, Torrvella), diphtheria (Kaufmann, Goodman) and in cholera. During the course of the severe systemic disease, the vaginitis is rarely noted unless a fould discharge attracts attention. More often the condition is first recognised at autopsy or after recovery, when it may evidence itself (Frank, 1931). True diphtheritic vaginitis is a necrotic process and may end as a gangrenous vaginitis, which may even become a paravaginal infection. This condition has been well described by Van Saun (1923), who records four deaths among twenty-seven cases in children. In the course of agranulocytic angina, grey patches may appear on the vulva and cervix, as in the fatal case of Skiles (1925). Arnold (1930) has reported a case of Vincent's disease of the vagina, which appeared subsequent to a throat infection. Agranulocytosis was present. These cases are exceedingly rare. Discharge may result/

result from ulceration of the vulva of the variety described by Welander (1903) as veneroid ulcer and by Lipschütz (1905) as ulcus vulvae acutum. This variety of ulceration is mainly found in virginal women — children and young adults — and tends to appear quite suddenly on the inner surface of the lacia majora and minora. A few cases of actinomycosis of the vulva have been recorded (Lieblein and Bongartz, Trapl, Kaplan). The infiltration, abscesses and fistulae associated with the disease are generally present. The discharge is purulent and contains the yellow granules characteristic of the "ray fungus." Membranous vaginitis is a rare condition resembling membranous enteritis and membranous dysmenorrhoea, superficial moulds being repeatedly cast off (Gellhorn, Kerwin). Vaginal ulcers are stated to result from any causes which produce localised defects in the epithelial covering of the wall. Much confusion has arisen in their classification. Uraemic ulcers, described by Eichhorst (1912), may be encountered in severe cases of uraemia. The

ulcus/

ulcus rotundum, which appears as a single or multiple punched-out lesion, may be partly due to arterio-sclerosis, but infection later undoubtedly enters into the process. (Frank, Fluhmann[1], 1929). Condylomata acuminata, although most commonly gonorrhoeal, are stated to be not necessarily venereal, resulting also from irritation of a non-specific nature, so that they may be found in children and virgins (Smith, 1903). Tuberculosis of the uterus or vagina in virgins may cause leucorrhoea. In the uterus it is rarely primary, and most frequently direct infection from the tube occurs (Murphy, 1903). According to White (1917), the uterus is affected in 53 per cent. of cases of genital tuberculosis and this is exceeded only by the Fallopian tubes 85 per cent. He also states that in only 2 per cent. of cases of uterine tuberculosis the cervix is alone involved. Fewer than 20 primary cases of cervical tuberculosis have been recorded (Douglass and Ridlon, 1929). Ulceration frequently develops early, the ulcers being undermined

and/

and dirty. A papillary type also occurs. The frequency of the various types, in 77 cases, has been described by Chaton (1908) as follows: ulcerating, 48; proliferative, 22; and miliary, 7. In uterine tuberculosis, the age group most affected is 20-30 years, but there are reports of infants of 7 and 9 months (Schlimpert, 1911) and of 44 cases under 15 years of age (Brüning, 1902). Primary tuberculosis of the vagina is very rare but may be the source of a slight discharge of sero-pus, generally not profuse. The condition has been fully described by Barnes (1930). Tuberculosis of the vulva is also very uncommon and Frank states that a primary lesion on this site has never been satisfactorily demonstrated, although Krömer (1907) considered his case as such. Tuberculosis of the vulva is commonest in early adult life, but von Kerajan (1897) has noted the condition in a child of two and a half years. A tubercular perineal abscess, opening into the vagina, the hymen being intact, may occur.

Carcinoma of the vulva usually occurs in women/

women over 50 years of age, but Kinoshita (1907) observed it in a virgin of 15 years. Lutzenberger found that eleven of 106 vulvar cancers occurred in nullipara or virgins. In the course of a review of the records of the Royal Samaritan Hospital for Women, Glasgow, over a period of ten years (involving over 23,000 admissions), the youngest virginal case of vulvar cancer which I encountered was aged 25 years. Carcinoma of the hymen in a virgo of 57 years is described by Frankl (1914). Cancer of the vagina is rare in nullipara, but has been encountered in virgins. A thin, watery, irritating discharge, is usually the earliest symptom and may be of considerable duration. Many isolated cases of cervical carcinoma in young women have been recorded. Aguinaga (1925) has described a vegetating cancer of the cervix in a girl of 14 years and Bonner (1927) has described a basal cell cancer in a girl of 12 years. In Lynch and Maxwell's series of 107 cases of cervical carcinoma with 5-year end results, there were several virginal cases described./

described. Eden and Lockyer (1920) describe the extremely rare condition of simple benign adenoma of the cervix and cite the only case in their experience. The women had suffered from profuse leucorrhoea from childhood. Intermediate between inflammation and benign and malignant neoplasms of the vagina, is the condition reported by Plaut (1928) under the designation of diffuse adenosis of the vagina. The entire vaginal canal was covered by a purple-red eroded mucosa. A similar condition was described in 1910 by Bonney and Glendinning as adenomatosis vaginae. With the exception of the Trichomonas vaginalis, vaginal parasites as a cause of irritation and discharge are not of much importance. Oxyuris vermicularis may be found, especially in the vagina of neglected infants (Chandler, 1918). Amoeba urogenitalis, Ascaris, Echinococcus and Filaria Bancrofti rarely occur.

Inflammation of the virginal vagina, due to the ingress of the common pathogenic organisms, and unassociated with the specific fevers already described,/

described, is generally believed to occur occasionally. Exclusive of cases in which a primary infection of the cervix co-exists or in which the causative factor is the Trichomonas vaginalis, it is my belief that a primary virginal vaginitis is rare. The organisms which one would expect to gain access most frequently to the vagina, in the event of a breakdown of the normal defensive mechanism, are naturally those which are most commonly present in the external genitalia. The organisms are mainly of intestinal origin and their number and variety depend to a great extent on the observance of cleanliness of the parts; staphylococcus albus, diphtheroids, coliform organisms, enterococcus and streptococcus viridans, micrococcus tetragenus and yeasts have all been isolated. In the vulva, a modified bacterial flora exists and organisms are less numerous (Cruickshank and Cruickshank). Fusiform bacillae and spirochaetes are frequently present about the clitoris (Pilot and Kanter, 1923)/

1923) and may play a secondary part in inflammatory conditions and ulcerations about the vulva. But it is difficult to conceive that any of these organisms can, of themselves, enter and flourish in the highly acid, inimical healthy vagina and produce a primary vaginitis. There can be little doubt that amny of the cases, which come under clinical notice from time to time, and have been considered cases of primary bacterial vaginitis, have really been cases of infection with Trichomonas vaginalis or with the Monilia fungus. These will be more fully discussed in subsequent pages. A radical extirpation of uterus and ovaries is occasionally followed by a thin, distressing leucorrhoea of vaginal origin. The condition is almost certainly due to changes in the epithelium consequent on oophorectomy and is sometimes extremely difficult to relieve.

With the exception of gross, local, pathological lesions such as fibroids and submucous polypi, an exhaustive account of the/

the rare etiological factors which may be concerned in the production of leucorrhoea in virgins has been given above. The results of my clinical and laboratory study and investigation, extending over three years, of some 40 cases of this condition will now be presented.

Original Investigations.

Each case of virginal leucorrhoea encountered has been examined in detail, both from the clinical and laboratory standpoints, but it is obviously undesirable that a mere series of descriptions of each of these cases should be given. The main features investigated appear in the description of the results. While I have investigated more than 40 cases, in a few of them observations have not been made over a sufficiently long period of time, on account of patients defaulting, or laboratory findings have been incomplete or unverified and so the described series is restricted to 35 cases. It is divided into two main categories:
(1) Non-infective and (2) infective. Details of

all/

all the cases appear in appendices C and D, but a more concise account follows here.

Among the non-infective cases there is a group in which hormonal disturbance is strongly suggested, either by the individual's"make-up" or by gross menstrual dysfunction such as prolonged or almost complete amenorrhoea. In other cases, there is no obvious evidence of endocrine imbalance and no distinctive clinical features are noted. The hormonal group are frequently excessively stout, sometimes complain of pain in one or other side of the abdomen and have lengthy spells of intermittent amenorrhoea during which leucorrhoea is often increased. Spontaneous remission tends to occur at intervals. The average age at onset is low, in my cases being 19. Sometimes the complaint dates from puberty, and in 3 of the series, discharge was troublesome before menstruation first commenced. No predisposing or exciting causal factors are apparent. Anaemia, debility, and constipation are not in themselves causes. In an attempt to find/

find evidence of endocrine dysfunction, skiagrams of the sella turcica, blood-sugar curves, basal metabolism rates and ZondekAscheim pregnancy tests have been made in a number of cases. In 2 of the 5 cases skiagraphed, the radiologist reported marked diminution of the sella turcica, in one slight diminution, one was unsatisfactory and the fifth was normal. Examination of the visual fields in these cases showed them normal. Blood sugar curves have been done in 4 cases and these have proved to be within normal limits, with the exception of one case in which the figure rose above 0.2. In this latter case, the basal metabolism rate was -20, there was slight glycosuria and it was thought that she was a potential diabetic. Basal metabolism estimations in the other cases have been found to be normal. Zondek-Ascheim tests have been uniformly negative.

The discharge is thick-white, inspissated or cheesy and non-irritating. It is not sufficiently fluid to pour forth out/

out of the vagina as is the case with a purulent discharge. It may be increased in amount pre-menstrually.

On vaginal examination, no lesion inflammatory or otherwise is discoverable and apart from the obviously excessive character of the secretion, there is no local evidence of anything abnormal except that occasionally the uterus may be much underdeveloped or the endometrium may show glandular hyperplasia. Bacteriological examination reveals information as follows: vaginal smear shows a Grade I flora - abundant B. Döderlein and epithelial cells and no pus cells; vaginal culture, on serum agar, a growth of B. Döderlein, or no growth whatsoever, or a few colonies of staphylococci; vaginal culture on Sabouraud's medium (for cultivation of yeasts), no growth of "yeasts;" cervical culture is generally sterile, but occasionally shows a few staphylococci, probably contaminant; intra-uterine culture (swab being passed through a hollow, tubular, glass, cervical dilator) is invariably sterile. Examination of the reaction of/

of the secretion demonstrates it to be highly acid, varying from pH 4.2 to pH 4.7. The following brief clinical histories exemplify some of the features of cases of the non-infective group:

Miss M.B., aged 23 years, was admitted to the Samaritan Hospital on 7.5.28, complaining of an excessive white discharge of about 7 years' duration and of dysmenorrhoea of 6 months' duration. On examination, she proved to be in good general health but excessively stout, weighing nearly 13 stone. Under anaesthesia, the hymen was found intact and no abnormality was detected in the vagina, cervix or pelvic organs. The cervix was dilated and the uterus curetted. The source of her leucorrhoea was not apparent. I saw her twenty months later, when she reported that her menstrual pains were considerably improved but that her discharge was undiminished. Radiostoleum (a preparation of Vitamins A and D) was prescribed for two months, but at the end of this time no appreciable improvement was noticed. Fifteen months later she informed me that discharge was still troublesome and that dysmenorrhoea had recurred. She had continually received attention to her general health. She was then re-admitted to hospital. Under anaesthesia, she was examined very carefully and no signs of infection could be detected in vagina or cervix. The latter was dilated for purposes of investigation. Both uterine and cervical cultures proved sterile. Vaginal smears showed a Grade I flora, and culture gave an almost pure growth of Döderlein's bacillus. The reaction of the discharge was highly acid – pH 4.2. It became obvious that her leucorrhoea was of the "non-infective" type. I gave her Emmenin (Collip's placental hormone, kindly supplied to me by Professor Collip, during a visit to Montreal), for four/

four weeks without any appreciable effect. It was then thought that irradiation of the ovaries, with a dosage short of a castration one, might prove of value and this was done by Dr. Bruce McLean. After two months, the patient reported some improvement in her complaint, but also menstrual irregularity. The improvement was not sustained and it was decided that the risks of irradiation outweighed any possible advantages. Negative results were obtained in the various lines of investigation mentioned previously, (basal metabolism, etc.), with the exception that radiography of the sella turcica showed it to be markedly diminished in size. Examination of the discharge again revealed a Grade I flora; the reaction was pH 4.4. Several "ovarian-hormone preparations" were subsequently tried for eight months, without any striking success. I saw her again in September 1932 when she stated that the condition was not quite so troublesome, but that, acting on my advice, she had come to regard it as her normal discharge.

Miss B., aged 13 years 9 months, attended the Out-Patient Department of the Samaritan Hospital, complaining of an excessive vaginal discharge. Menstruation had not yet occurred. There was some evidence of puberty in slight development of the secondary sex characteristics. Her own doctor had mentioned the possibility of the discharge being gonorrhoeal in origin and accordingly the patient's mother was greatly concerned. Clinical examination revealed an intact hymen, no obvious signs of infection, but an obvious leucorrhoea. Bacteriological examination of the discharge showed a pure, Grade I flora and the estimation of its reaction showed a pH of 4.6. The condition was obviously of the "non-infective type" and no treatment, other than that of a general medical type, was suggested. At the end of six months, the patient reported, considerably improved.

The other category is the infective one and in these cases the age incidence tends to be higher. In this group, the majority of the cases have been Trichomonas vaginalis infections, while one definite case of yeast or Monilia vaginitis has been encountered. These conditions are fully described in subsequent pages, but a few brief remarks on them may be made here. Nine cases of Trichomonas infection in virgins have been studied. As compared with the non-infective group, the discharge is more watery, yellowish, finely frothy, occasionally blood tinged and generally irritating. The vulva and vagina frequently appear to be inflammed. Diagnosis of the condition can only be made by fresh-drop examination of the secretion. A vaginal smear shows a Grade III flora – abundant, mixed organisms and pus cells – and vaginal culture yields a variety of organisms, but no Döderlein bacilli. The reaction of the secretion varies from pH 4.9 – pH 6.0. In the single case encountered of Monilia vaginitis the discharge was profuse and semi-purulent, causing considerable irritation and extensive intertrigo. On fresh-

drop/

drop examination and smears, abundant Monilia or yeast-like bodies were seen. In only two cases of the series did it seem as if an "erosion" of the cervix was the sole cause of leucorrhoea. A typical case of the infective type of virginal leucorrhoea is as follows:

Miss R., aged 23 years, was admitted to the Samaritan Hospital on 9.8.29, complaining of a profuse, irritating discharge of three years' duration. Under anaesthesia, the hymen was intact and no pelvic abnormality was detected, but the cervix was dilated and the uterus curetted. She reported, four months later, that there was no improvement. Various attempts at douching were made and several "ovarian preparations" administered. She was re-admitted to hospital on 12.3.30. The cervical canal was cauterized and the vagina douched three times daily for ten days. Vaginal smears showed a Grade III flora. Her condition improved for two months but one month later she reported that discharge was "as bad as ever." Radiostoleum was exhibited for one month and there was an apparent improvement, which proved temporary. Fresh-drop examination of the yellowish, finely-frothy discharge was then made and revealed abundant Trichomonas vaginalis. Intensive local treatment against this parasite was then carried out, and leucorrhoea improved considerably.

The laboratory findings in the two categories of cases of virginal leucorrhoea are summarised in the following table:

	Non-Infective.	Infective.
Secretion.	Whitish, viscid, cheesy.	Grey-yellowish, fluid, frothy.
Smear.	Grade I flora	Grade II or III flora.
Culture.	B. Döderlein.	Profuse, mixed.
Reaction (average pH).	4.4.	5.6.

Observations on Etiology.

The laboratory findings in the non-infective group lead one to the conclusion that leucorrhoea in these cases is nothing more or less than an excessive production of the normal discharge of the vaginal epithelium. Support for this view is present in the analogous processes occurring in the later months of pregnancy, as described by Cruickshank and Baird (1930), and in neo-natal life, as demonstrated by myself earlier in these studies. Cruickshank and Baird have shown that as pregnancy advances, the vaginal flora/

flora tends to improve from a Grade III to a Grade I, and simultaneously the amount of vaginal secretion increases. The correlation of these changes to the presence of glycogen in the epithelium and to ovarian activity has already been pointed out. Again, the changes in vaginal secretion from abundance to scantiness during the first 3 weeks of life and from scantiness to a moderate quantity at puberty, have been shown to be intimately correlated with the reaction and bacteriology of the secretion and with the thickness and glycogen content of the epithelium. One arrives at the opinion therefore, that in many cases of virginal leucorrhoea (non-infective group), the excessive discharge is simply a marked excess of vaginal secretion, probably associated with excessive deposition and conversion of glycogen in the vaginal wall, which in turn is dependent upon ovarian control. The precise nature of the hormonal disturbance responsible is not known, as it is considered very difficult and the results unreliable for anyone other than an expert to assay accurately excess or deficiency/

deficiency of the ovarian hormone. I believe that the process is not analogous to the excessive mucification, due to corpus luteum hormone, seen in the vagina of many lower animals, as the mechanisms in the human and animal vaginal epithelia are quite different. However, I have endeavoured by investigations already mentioned (skiagrams, etc.) to find evidence of endocrine dysfunction. The tendency to marked obesity in some of the cases is suggestive. Skiagrams of the sella turcica, in 2 cases out of 5, revealed marked diminution in its size. Ragúz, Haro and Villaverde (1932) in recording observations on the size of the sella in forty-nine patients, state that in women with amenorrhoea it is larger than normal, but that variations in its size cannot be regarded as possessing an absolute value. Zondek-Ascheim tests (for excess of anterior pituitary hormone in the urine) have proved negative. Mazer and Hoffman (1931) have found 7 positive reactions among 38 women with ovarian hypo-function and Fluhmann[2] (1929) has found/

found anterior pituitary hormone in the blood of 12 of 19 patients who had a previous bilateral oophorectomy, and in the blood of 6 of 17 women suffering from functional amenorrhoea. Blood sugar curves and basal metabolism estimations in my cases have yielded negative results. Raab (1931), however, has shown that, under the influence of ovarian follicular hormone, extracted from the actual follicle, sugar introduced into the blood stream in women disappears from it more rapidly than is normally the case. In animal experiments he has demonstrated that the liver of animals, which had been injected with follicular extract, contained two and three times larger amounts of glycogen than the liver of animals which had not been injected. He concludes that "ovarian hormone" effects a very considerable formation and fixation of glycogen in the liver. These results of Raab's are suggestive of an intimate metabolic relationship between the ovarian follicle and glycogenesis in the vaginal epithelium.

Consideration/

Consideration of the infective group of cases is now necessary, and, firstly, some observations on "erosion" of the cervix will be made. It is my experience that, in virgins, an erosion is only occasionally the cause of the complaint of leucorrhoea. Frequently, erosions are seen in virgins who are operated on for other symptoms, but who have no complaint of excessive discharge. Again, I have repeatedly seen cases, in the course of a "follow-up", in which an erosion, the presumed cause of leucorrhoea, has been cauterised or excised without any appreciable improvement in the discharge, although subsequent examination has revealed no evidence of the original erosion. It is to be noted, too, that in the cases of the series in which it has been done, cervical culture has proved almost invariably sterile. In the non-virgin, on the other hand, in cases of chronic cervical infection, organisms are frequently present, e.g. in the series of 40 cases of Barrett, Lash and Pilot (1925), who recovered streptococci in 40 per cent. of them. Curtis (1929),/

(1929), in a personal communication, has stated his belief, "cervical infection is of minor "importance in virginal leucorrhoea, but the "trouble begins with an emotional increase in "the gland secretion, followed by maceration of "the tissues and resultant hyperplasia of the "glands: a vicious circle is thus established "with the result that there is a continuous "outpouring of secretion." The role of the cervix in the production of a discharge of doubtful origin may be investigated by estimating the reaction of the latter. The cervical reaction is constantly and definitely alkaline and, according to Meaker and Glaser, ranges from pH 8.0 to pH 9.0. Its pH is above 8.5 in about 80 per cent. of cases and is not notably influenced by age, parity or endocervicitis, notwithstanding the fact that in the last-named condition a great increase of secretion may take place, that it may be mucoid, muco-purulent or blood-tinged, and may contain pus cells, lymphocytes, epithelium and bacteria. Accordingly, these facts serve further to/

to convince one that the alkaline discharge of cervical origin is readily differentiated from the highly acid vaginal one, and that the former cannot be present sufficient to produce a leucorrhoea, the reaction of which is, for example, pH 4.4. Infection of the uterine cavity, as a source of leucorrhoea, is extremely rare. Curtis (1918) has conclusively proved this by histological and bacteriological examinations of the endometrium in a large number of cases.

To conclude the study of the cases of the infective group, Trichomonas vaginalis vaginitis and Monilia vaginitis remain now to be described.

Trichomonas Infection of the Vagina.

During recent years, considerable attention has been paid to the Trichomonas vaginalis as a pathogenic protozoon and as a cause of leucorrhoea. A considerable literature has appeared, particularly in the United States since Greenhill's paper in 1928. The parasite has,/

has, however, been known for almost one hundred years, having been first described by Donne in 1836. Its presence in the vaginal secretion of both pregnant and non-pregnant women has been subsequently confirmed by numerous investigators. In 1855 Koelliker and Scanzoni found the organism in the yellowish, highly acid discharge in 50 per cent. of pregnant and non-pregnant women. They were unable to demonstrate its presence in the so-called normal secretion. Haussmann in 1870 reported similar observations and asserted that the associated discharge was in most cases foamy or frothy and highly acid in reaction. He found the flagellate in 40 per cent. of non-pregnant women in whom pelvic disease was present. In 1913 Brumpt discovered Trichomonas in the vagina in 10 per cent. of all patients whom he personally examined. The most important clinical work on this protozoon was done by Hoehne (1916), who stated that, although he found it in 28 per cent. of non-pregnant women, in most cases it was present in very small numbers. He, like the other/

other authors quoted, stated that it was present only in women in whom the vaginal secretion was abnormal. Wille (1918), in an analysis of a thousand patients suffering from various forms of gynaecological conditions, reported that Trichomonas was found in the vaginal discharge of 40 per cent. of those who complained of an annoying type of leucorrhoea. Traugott (1918), in a study of the vaginal secretion of non-pregnant women suffering from cervical disease, discovered the parasite in 50 per cent. of those whom he personally examined. He thought that lesions of the cervix seemed to predispose to Trichomonas invasion. Reuling (1921) observed it in 18.4 per cent. of 250 cases whose chief complaint was leucorrhoea. In order to determine the presence or absence of Trichomonas infection in the normal vaginal secretion, as found in young individuals, Ponoschina (1923) conducted an investigation of 22 girls, ranging in age from 2 to 14 years. In none of these was the parasite isolated. Flaskamp (1925) reported/

reported that not more than one-third of the patients harbouring the flagellate complained subjectively. Neumann and Mayer (1914), Hegner (1925), Schmid and Kamniker (1926) and Vavilova (1927) studied several series of cases and found a varying incidence of Trichomonas.

In 1920, De Lee, in 1928, Greenhill and in 1929, Davis, diagnosed a specific condition which they designated Trichomonas vaginalis vaginitis. Since 1929 there have been reported many series of cases of this condition, among the most important publications being those of Andrews (1929), Furniss (1930), Kleegmann (1930), Brooke Bland, Goldstein and Wenrich (1931), Greenhill (1931) and Goodall (1931). These authors are all convinced of the pathogenicity of the parasite. They describe the clinical condition and its treatment. There is, however, no conclusive proof of the definite pathogenicity of this flagellate. Neumann and Mayer (1914), Wölfring (1921), Loeser (1922) Haupt/

Haupt (1924) and Seeliger (1927) maintain that it is a harmless parasite. Most other investigators, however, regard it as a specific cause of a vaginitis, although it may occasionally be encountered in the vagina in scanty numbers as non-pathogenic. Flaskamp describes it as being "relatively pathogenic." Brooke Bland, Goldstein and Wenrich find sufficient proof of its pathogenicity in that, firstly, numerous papers have been published supporting this view; secondly, that it may be transmitted from one individual to another; and thirdly, because rather characteristic morbid changes occur in individuals in whom it is transferred.

The morphology of Trichomonas is now fairly well known. It is best studied by means of hanging-drop preparations or by diluting a drop of vaginal secretion with a drop of normal saline on an ordinary glass slide. The drop usually consists of a large number of leucocytes and bacteria, many epithelial cells and many Trichomonas. With the ordinary high-power lenses, the/

the parasites may readily be seen because of their motility. Stained smear preparations do not show the organism clearly, but with a little experience of its appearances, one can detect it when stained by Gram's method or with Heidenhahn's iron haematoxylin. The Trichomonas vaginalis varies considerably in size, being usually larger than a polymorphonuclear leucocyte but smaller than an epithelial cell (Greenhill, 1931). The measurements given by various authorities vary, as for example, length 7 to 21 u, width 6 to 18 u (Hegner), and length 10 to 30 u, width 10 to 15 u (Reuling). It also varies in shape, being usually pyriform, fusiform or pear-shaped. (This is illustrated in the sub-joined outline drawings).

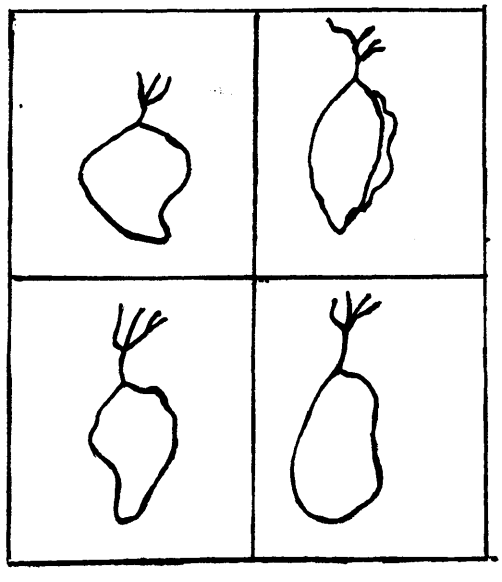

Fig. 29. All the organisms have four flagella, and one shows the undulating membrane. (From Greenhill).

The front end is rounded and from it protrude four flagella which arise from a common stem. The flagella are approximately of the same length and are more than half as long as the body of the organism. Commencing from a dark granule near the base of the flagella, an undulating membrane runs somewhat spirally along the entire body to the posterior end which is usually pointed. The nucleus of the Trichomonas is usually eccentrically situated near the base of the flagella and is oval or pyriform in shape. In fresh material, the living flagella are exceedingly active. They may exhibit a jerky forward motion, accompanied by a counter-clockwise rotation on their long axes, or they may, if surrounded by cellular debris, "crawl" their way through such material by assuming a great variety of shapes. The locomotion of the organism is independent of the flagella but is dependent upon the undulating membrane (Greenhill). During continued observation on a slide, progressive degeneration takes place, and the Trichomonas

may/

may round itself up and eventually simulate a cyst (Brooke Bland, Goldstein and Wenrich). Without any special precaution, it can be kept alive at room temperature for three to four hours. It nearly always lives in symbiosis with other organisms, usually bacteria and most often the latter are Gram-negative cocci which are smaller than gonococci. In most cases the bacterium found is the micrococcus aerogenes alcaligenes, which according to Loeser and others, is responsible for the small gas bubles which are almost constantly found in the discharge. The association of the gonococcus and Trichomonas is very rare (Greenhill). Hoehne states that he has never encountered this dual infection, but cases of this nature have been reported by Loeser, Flaskamp, Seitz (1919), and Andrews. Cultures of Trichomonas vaginalis are difficult to make. Lynch in 1915 was the first to succeed in obtaining a growth in beef broth. Several different media have since been used, but their essential nature is that of saline solution with some nutrient material such as fresh or dried serum

or/

or ascitic fluid.

The origin of this vaginal flagellate is unsettled. A Trichomonas is frequently encountered in the mouth and in the faeces of normal persons, but the vaginal type is stated by some writers to have recognisable differential features. Bensen (1910) and Lynch (1922), however, believe that the three types belong to the same species. Hegner believed that he was able to transfer Trichomonads of the monkey to the vagina of monkeys of the same species and therefore believed that the flagellates from the two parts of the host were identical and suggested a like possibility for man. Andrews has recently stated that in long continued cultures, Trichomonas vaginalis may change so as to resemble Trichomonas hominis.

The clinical picture in Trichomonas vaginalis vaginitis is uniform and striking. The patient complains of a profuse purulent discharge, frequently associated with irritation or a burning sensation in the vagina or vulva. The/

The discharge is generally yellowish, often offensive in odour, sometimes watery, almost always finely frothy and may resemble gonorrhoeal pus very closely. The introitus and the vaginal mucosa are characteristically reddened, a punctate hyperaemic vaginitis being present.

Very little reference has been made in the literature to the occurrence of this protozoon in virgins, but, in a personal communication, Greenhill (1930) has informed me that he has found the parasite "not infrequently in virgins." Davis (1929) has also informed me that among 73 private cases of Trichomonas infection, during a period of 22 months, he was "relatively certain of virginity in 4 cases." Furniss has recovered it in a girl of 12 and states that in his series of 35 cases of Trichomonas infection, 3 of the patients were "undoubtedly virgins." Brooke Bland (1931) has described the case of a woman of 34 years of age who had treatment for vaginal discharge over a period of 32 years, i.e. since the age of 2 years, when/

when she had developed what had been regarded as a gonorrhoeal infection. After more or less constant treatment, of all manner, during 32 years, it was discovered that the condition was a Trichomonas vaginalis vaginitis. Personally, I have no doubt that Trichomonas infection of the virginal vagina occurs and that it is definitely more common than is generally supposed, even by experienced gynaecologists.

Numerous forms of treatment have been suggested but no specific or wholly satisfactory therapy has yet been found. Greenhill advocates a combined "green soap - methylene blue - glycerine - lactic acid" technique. Brooke Bland and his associates use, in addition, 1 per cent. compound solution of cresol as a cleansing agent and iodine solution as a douche. Davis prefers 5 per cent. mercurochrome or liniment of soft soap, and Furniss advises 1-4,000 perchloride of mercury as a twice-daily douche. Goodall believes that 1 per cent./

cent. Picric acid is the best antiseptic to employ and regards it as being almost specific for the organism. Prolonged treatment by any of these methods, extending over several weeks may be necessary, particularly in cases of long duration.

Fungus Infections of the Vagina.

Fungus infections of the vagina are distinctly uncommon and are rare in virgins. There is no doubt, however, that their presence has been frequently overlooked and the true nature of a vaginitis mis-diagnosed.

The presence of yeast-like organisms in the vagina was known long before the work of Döderlein and his contemporaries. According to Castellani, Wilkinson in 1840 (Lancet I), described them in vaginal discharge. Mayer in 1862 reported six cases and he was followed by others who recorded occasional "infections." They were described more fully by von Winckel in 1866 and by Haussmann in 1875. Cases of vaginal thrush were described by Kleemann, Mettenheimer, Giulini and Panfilowicz, all prior/

prior to 1892. Further descriptions were published by von Herff (1894), by Colpe (1894) and by Littauer (1905), and during the last thirty years an extensive literature has accumulated. Of recent years, doubt has been cast by some on the etiological importance of yeast-like fungi in producing vaginitis. Most of the leading text-book authors describe the fungus as being pathogenic, e.g., Graves and Frank. Recent reports in the literature support this view, e.g. the publications of Le Blaye, Moench, Davis, Heard, Popoff, Ford and Cadmus, Odland and Hoffstadt, Flusser, Perazzi, and Cordey. Castellani and Taylor (1925) state that, whereas in normal vaginal secretion yeast-like bodies are absent or extremely scanty, in vaginal discharge the round or oval cells are frequently observed. As far as the skin is concerned, numerous inoculation experiments have proved that certain monilias are definitely pathogenic. McLeod (1930) states that this has been demonstrated by himself and others with M. albicans and M. pinoyi. His account of the Monilias/

Monilias briefly summarises present-day knowledge of them and is worth quoting in part. "They "belong to the family of oosporaceae, reproduce "themselves by free spores or conidia and present "budding forms on cultivation on artificial media. "The mycelium shows hyphae, which are usually long "and septate. In culture, the fungi are gram-"positive, liquefy gelatin and ferment certain "sugars such as glucose and maltose, with in the "case of glucose the formation of gas. They grow "readily on Sabouraud's maltose agar (pH 5.5) at "25°C. in from 24 to 48 hours. There are several "varieties of monilia, certain of which are "pathogenic, while others are only sabrophytic. "Differences can be detected by means of bio-"chemical tests, based on their capacity of "fermenting different sugars. The most familiar "member of the group is Monilia albicans (Oidium). "Other well-known pathogenic monilia are M. pinoyi "and M. candida."

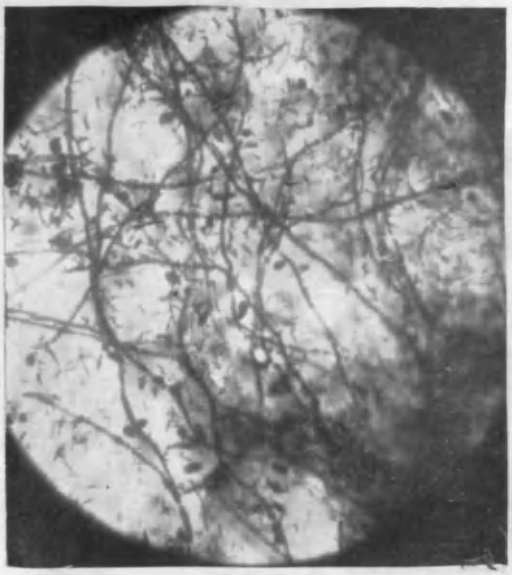

Fig. 30 - Monilia in vaginal smear.
Photomicrograph, high-power.
(from Plass, Hesseltine & Borts).

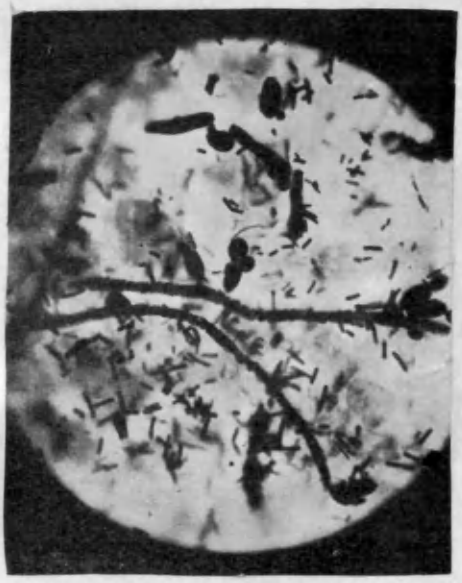

Fig. 31 - A microphotograph (oil-immersion) showing mycelia and conidia.
(from Plass, Hesseltine & Borts).

Fig. 32 - Monilia in culture. Many bud forms but few mycelial threads. Photomicrograph oil-immersion. (from Plass, Hesseltine & Borts).

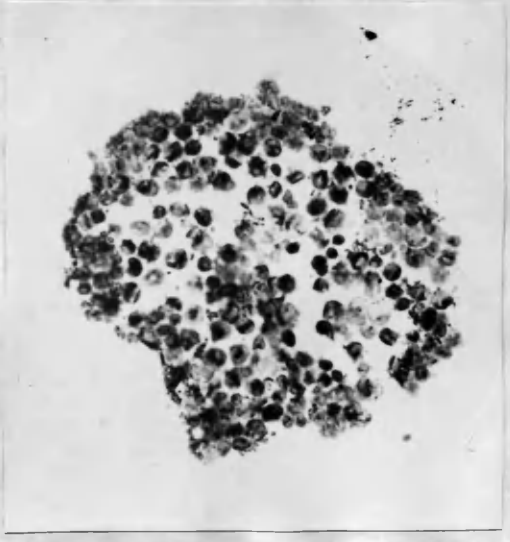

Fig. 33 - Monilia in culture, from case of "virginal leucorrhoea" - Miss Halcrow.

That vaginal monilias were non-pathogenic was the view expressed by Zweifel in 1895, and, comparatively recently, Stephan (1925) has upheld this opinion. He states that the fungus is not really the important etiological agent in producing vaginitis but that by excoriating the mucosa it permits the entrance of a profuse, mixed bacterial flora, which actually causes the inflammatory process. Experimental work in demonstrating the pathogenicity of vaginal monilias has not been convincing. Von Winckel (1866) inoculated the vaginas of rabbits, but produced only slight reddening of the mucosa. Colpe (1894), similarly, noted diffuse congestion and a serous discharge. Two weeks after inoculation, the fungi could not be found in the vagina. Haussmann (1875) inoculated a pregnant woman, whose vagina was fungus-free, with material taken directly from the vagina of a case of mycotic vaginitis. Ten days later, vaginal burning and vulvar irritation appeared. These subsided spontaneously, but recurred at intervals until active treatment was employed to destroy the organisms./

organisms.

Castellani (1929) has found the following genera of fungi in the vagina: Monilia, Saccharomyces, Cryptococcus, Oidium, Aspergillus, Penicillium, Hemispora and others. He believes that they act only as saprophytes when present in small amount, but, when present in large numbers, he is practically certain that they produce inflammation. On the whole, the available evidence leads one to the view that Monilia, while not proved conclusively to be pathogenic, may occasionally be the cause of a definite, specific vaginitis, which may be associated with an increased amount of vaginal secretion or even a profuse, purulent discharge. The situation is quite comparable to that which exists regarding Trichomonas vaginalis.

The types of organism vary. Von Herff, in 22 cases, found Monilia albicans sixteen times, M. candida four times, Leptothrix vaginalis and an unidentified yeast-like fungus once each. Castellani and Chalmers (1919) have described seven varieties of/

of Monilia. Popoff, Ford and Cadmus (1929) identified the organism in their case as M. psilosis ashfordi, and Odland and Hoffstadt (1929) identified theirs in a married woman as M. pinoyi. The incidence of yeasts and of actual mycotic vaginitis in pregnant women is relatively high, but no figures seem available for the non-symptomatic occurrence of the fungus in non-pregnant women. Wille (1918), in an analysis of 1,000 patients suffering from various forms of gynaecological conditions, and of whom 183 complained of an annoying type of leucorrhoea, reported an Oidium albicans infection in 2.

Monilia vaginitis most commonly affects multiparous women, with pregnancy certainly and diabetes very probably predisposing factors, but cases have been recorded of infections in virgins. Plass, Borts and Hesseltine (1930) state that presumably the increased acidity of the vaginal secretion during gestation favours the growth of Monilia. Döderlein had, of course, noted this in 1892. Mettenheimer[1]/

Mettenheimer[1] (1882) and Flusser (1929) both record cases, one a child of seven years, in whom vulval infections followed angina due to the fungus. Menge and Opitz (1913) state that children are occasionally infected. Mettenheimer[2] (1882) reports an infection in an adult virgin. Castellani (1926) describes a case of an elderly spinster who had an abundant purulent vaginal discharge, which, on examination, revealed enormous numbers of monilias (M. pinoyi, Castellani).

Clinically, pruritus may be the only symptom, but frequently profuse vaginal discharge is present. Dysuria or dyspareunia may be present. Castellani (1929) describes three clinical types, associated with vaginal moniliasis: (a) membranous type or "vaginal thrush;" (b) purulent type, in which discharge is thick and yellowish and may be mistaken for gonorrhoea: (c) a mixed type, in which thrush-like patches are present and the discharge is purulent and thick. Stephan (1925) claims that all his patients had had a noticeable leucorrhoea. There/

There is no convincing evidence that monilia can infect the cervical canal. The introitus vagina and the vaginal mucosa are frequently diffusely reddened and there may be a marked granular appearance. Less commonly, thrush-like patches may be present, resembling oval thrush or vaginal diphtheria. Precise diagnosis is made by examining smears stained by Gram's method and cultures on Sabouraud's media. The branched, budding, mycelial forms are quite characteristic, but the conidia resemble leucocytes very closely. Monilia are sometimes associated with Trichomonas vaginalis.

Treatment has been attempted by various clinicians with all manner of local applications and douches. Alkaline douches have been strongly recommended. During very recent years, one per cent. aqueous gentian violet has been employed with considerable success (Cooke, Moench, Heard, Plass, Hesseltine and Borts). Faber and Clark (1927) state that gentian violet is the most effective of the common antiseptics and kills Monilia in a dilution of 1:25,000. Tanner and Bollas/

Bollas (1928) report that when incorporated into media, it will destroy the fungi at a dilution of 1:80,000. Relief or complete disappearance of symptoms after treatment sometimes occurs, although Monilia may still be found in small numbers in the vagina. Again, the condition may occasionally, without the local application of antiseptics, undergo spontaneous relief. But there can be little doubt that Monilia vaginitis is a definite clinical entity and that when symptoms are present, the abolition of the fungus from the genital canal is definitely indicated.

Treatment.

Treatment must depend on a careful examination of the discharge and, on this, a determination of the causative process must be made. The method of investigation employed and recommended is that a spoonful of the discharge is removed in a vaginal spoon (quite easily done in most cases without an anaesthetic) and fresh-drop examination in saline, smears and cultures and/

and pH estimations are made. In the infective cases, treatment has already been described and consists in the local application of antiseptics to the vaginal wall, generally in the form of instillations or douches. Particularly in Trichomonas cases, this must be very thorough, some of the best agents being tincture of green soap, picric acid 1 per cent., lactic acid ½ per cent. and Lugol's iodine. In persistent leucorrhoea which defies all types of antiseptics, brewer's yeast has been recommended as a vaginal inoculation (Abraham, 1910). Adler (1930) states that dry dusting does not give good results in the treatment of vaginitis. In the case of erosion, infective or simply hypersecreting, treatment is directed to its removal or destruction, e.g. by means of excision or cauterization.

With regard to the non-infective category, local treatment is not likely to influence the condition. Theoretically, hormone therapy might prove of value, but I have tried many preparations and am not satisfied with their efficacy in this direction. Numerous varieties

of/

of tonics and vitamin preparations have been given, but the results have been unsatisfactory. Bauer (1929), however, has treated ten cases of leucorrhoea in young girls and virgins with Vigantol (irradiated ergosterol) and claims satisfactory results. According to Palay (1931), who considers that leucorrhoea should be included among the vitamin deficiency disorders, a certain "percentage of his patients improved rapidly as the "result of vitamin therapy." It is well-known, of course, that the reaction of the intestinal contents can be altered by the food and that such alterations cause changes in the bacterial flora, but there is no evidence that a similar process can occur in the vagina. The question of the value of irradiation of the ovaries as a therapeutic measure in these cases must at present remain unanswered. There is considerable dispute over the stimulating effects of X-rays in small quantities to organs. However, in functional amenorrhoea of young women, in fifty per cent. of cases normal menstruation resumes after a 6-10 per cent. erythema dose applied to the ovaries./

ovaries (Kelly). It is very doubtful if the risk of irradiation justifies its use until the precise effects of varying shades of dosage on the ovaries are definitely established. General medical treatment may be employed and the nature of the condition may, with advantage, be explained to the patient. The tendency in non-infective cases to spontaneous remission and cure (the latter occurring frequently after marriage). must be taken into account in assessing the value of any treatment. The important point is that it should not follow along the therapeutical lines necessary in cases of the infective category. Dilatation and curettage is worse than useless, for, in addition to it being based on false premises, being of no value and hence unnecessary, it is occasionally followed by infection and even pelvic peritonitis, as in the case recorded by Frassineti (1929).

In conclusion, it may be said that virginal leucorrhoea is a condition very distressing to the patient and one which merits a thorough and/

and methodical investigation. In diagnosis and in deciding the method of treatment a careful clinical history and examination is of value, but a definite diagnosis can only be made by a full and careful laboratory examination of the discharge, along the lines which have been described.

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APPENDIX A.

INVESTIGATION OF VAGINAL SECRETION IN INFANTS (101 cases)

(The following abbreviations are used:

orgs., organisms; ent., enterococci; staph., staphylococci; alb., albus).

1st day.					2nd day.			4th day.			9th day.			6 weeks.		
Case No.	Age in Hours.	Smear	Culture.	pH	Smear	Culture	pH	Smear	Culture	pH	Smear	Culture	pH	Smear	Culture	pH
1.	10	Cocci (?)	Staph. (scanty)	5.6	?orgs.	Staph., ent.	5.6	Dod. ++	Dod. & staph.	4.8						
2.	4	orgs. –	Nil.	6.2 (?)	?orgs.	B. proteus etc.	5.7	Dod. ++	B. proteus etc.	4.8				Dod. staph.	B. pyocyan.	5.2
3.	16	Dod. ?	Staph. & ent. ±	5.6	Cocci ±	Staph. ent. +	5.6	Dod. ++	Dod ++ staph.	4.7	Dod. ++ ent.	Ent.	4.7	Scanty cells ? orgs.	Staph.	6.6 (?)
4.	12	orgs. –	Nil.	5.7	orgs. –	Staph. ent. +	5.6	Dod. ++	Dod. +	4.7				Scanty cells Dod. ±	Nil.	–
5.	3	orgs. –	Nil.	5.9	orgs. –	Staph. ent. ±	5.6	Dod. ++	Ent.	4.7	Dod. diph. ent.	B. proteus etc.	4.9			
6.	8½	Dod. ∓	Nil.	5.6	Dod. +	Ent. ∓	5.2	Dod. ++	Ent.	4.7	Dod. diph.	Pyocyans.	4.8			
7.	13	orgs. –	Nil.	6.0	Dod. ∓ Ent. +	B. proteus etc.	4.8	Dod. ++	Proteus and ent.	4.8	Dod. diph. & ent.	B. proteus	4.8			
8.	13	orgs. –	Nil.	6.1	Ent. ∓	Ent.	4.9	Dod. + ent.	Ent.	4.8	Dod.	Staph. (scanty)	4.8	Dod. ++	B. proteus.	4.9
9.	5	orgs. –	Nil.	?6.6	Dod. +	Scanty staph.	5.4	Dod. ++	Mixed, ?Dod.	4.8(?)				? orgs.	?	–
10.	12	orgs. –	Nil.	5.9	Dod. +	Dod. & staph.	5.9	Dod. ++	Scanty staph.	4.8	Leucocytes +, Dod., diph.	Scanty staph.	?(scanty secretion)			
11.	13	orgs. –	occas. staph.	5.7	Dod. ++	Scanty staph.	4.8?	Dod. ++	Dod. & ent.	4.7	Dod. + staph. +	Staph. aureus	4.9			
12.	¾	orgs. –	Nil.	Too scanty.	Dod. ∓	Mixed.	5.8	Dod. ++	Dod. & staph.	4.7	Dod. +	Staph. & ent.	? (too scanty).			
13.	7½	orgs. –	Nil.	6.0	Scanty orgs?	Staph., ent.	5.6	Dod. ++	Scanty staph.	4.7	Dod. ++	Staph., ent.	4.7			
14.	⅓	orgs. –	Nil.	5.7				Dod. ++	Scanty staph.	Not done.				Scanty Dod.	Staph? Dod.	6.5
15.	20	orgs. –	occas. staph.	5.7	Dod. + ent.	Staph., ent.	4.9	Dod. ++ cocci +	Staph., ent.	4.8	Dod. ++	B. proteus etc.	4.8	Scanty Dod.	Staph ± Dod. ±	6.8 (?)
16.	21	orgs. –	2 colonies of staph.	5.7										Dod.	Nil.	6.8
17.	22	orgs. –	scanty staph.	5.5	Scanty Dod.	Staph., ent. ∓	5.3	Dod. ++	Dod. +	4.8						
18.	4½	orgs. –	Nil.	5.7	?orgs.	Cocci in short chains.	5.2	Dod. ++	Dod. +	4.9	Dod. ++	Dod. +	4.8	? orgs.	B. proteus.	6.8
					?orgs.	B. protens etc.	5.6	Dod. ++	Ent.	4.8						

Case No.	1st day. Age in Hours.	1st day. Smear	1st day. Culture	1st day. pH	2nd day. Smear	2nd day. Culture	2nd day. pH	4th day. Smear	4th day. Culture	4th day. pH	9th day. Smear	9th day. Culture	9th day. pH	6 weeks. Smear	6 weeks. Culture	6 weeks. pH
19.	7	orgs. –	Nil.	5.9	? orgs.	Staph. & ent.	6.0	Dod. ++	Ent.	4.8	Dod. ++	Dod. +	4.8	Scanty Dod.	Scanty staph.	6.8 (?)
20.	5½	orgs. –	Nil.	5.8	Dod. ++	Scanty staph.	4.8	Dod. ++	Scanty staph.	4.7	Dod. ++	Nil.	4.7			
21.	6	orgs. –	Nil.	5.7	Dod. ++	Dod. +	4.7	Dod. ++	Ent. (?)	4.7	Dod. ++	3 staph. albus	4.7	Scanty Dod. & cocci.	Dod. + staph.	Not done
22	12	orgs. –	Nil.	5.6	? orgs.	Scanty staph.	5.5	Dod. ++	Dod. ++	4.8	Dod. ++	B. proteus.	4.9	Scanty Dod. & cocci.	B. proteus.	6.8 (?)
23.	14	orgs. –	Scanty staph.	5.4	orgs. –	Scanty staph.	5.6	Dod. ++	Scanty staph.	4.8	Dod. ++	Staph. & ent.	4.8	? orgs.	Nil.	–
24.	14	orgs. –	Nil.	5.8	Dod. +	Staph., ent.	5.7	Dod. ++	Scanty staph.	4.8				Scanty orgs.	Nil.	6.8 (?)
25.	16	orgs. –	Staph. +	6.0	? orgs.	Dod. +	5.5	Dod. ++	Scanty staph.	4.8						
26.	1¼	orgs. –	Nil.	5.6	? orgs.	Staph. & ent.	5.4	Dod. ++	Dod. +	4.8	Dod. ++	Dod. +	4.8	Scanty orgs.	Scanty staph.	6.8 (?)
27.	15½	orgs. –	Staph. albus +	5.7	Ent. +	Ent.	5.7	Dod. ++	Dod. ++	4.8	Dod. ++	Dod. ++	5.2	Scanty Dod.	B. proteus.	–
28.	14	orgs. –	Staph. albus +	6.4	Ent.	B. proteus active.	6.5	?Dod. or diph. ++	B. proteus etc.	5.7	? mixed	B. proteus etc.	5.2	(Slight red discharge - 4th day).		
29.	7	orgs. –	2 C. of staph.	5.8	Dod. ++	Dod. +	4.7	Dod. ++	Dod. +	4.7						
30.	22	orgs. –	Scanty staph. & ent.	5.7	orgs. –	Scanty staph.	5.7	Dod. +	Staph.	4.8	Mixed	Staph.	Nil	Scanty Dod.	Staph.	?
31.	11½	orgs. –	Nil.	6.0	Dod. ++	Scanty staph. & ent.	4.8	Dod. ++	Dod. +	4.7	(Dod. ++	Dod. (?)	4.8	Mixed Dod., staph., ent.	Scanty staph.	6.4
32.	20	orgs. –	Scanty staph.	5.8	Dod. + scanty ent.	Staph. & ent.	4.8	Dod. ++	Dod. +	4.7	Scanty orgs.	B. proteus	6.5 (?)	? orgs.	Staph.	?
33.	23¾	diploc.	?	5.0	Dod. & ent.	Ent.	4.8	Dod. ++	B. proteus	4.7	Dod. ++	Scanty staph.	5.4 (?)	Scanty Dod.	B. proteus.	?
34.	17½	orgs. –	Nil.	5.6	? orgs.	Staph. & ent.	4.8	Dod. ++	Ent.	4.8	Scanty Dod.	Dod. +	5.2			
35.	4½	orgs. –	Nil.	5.5	Scanty Dod.	?	5.4	Dod. ++	Dod. + ent.	4.7	Dod. ++ ent. + Dod. ++	Dod. +	4.7	Mixed pus cells ++, B. proteus		5.9
36.	½	orgs. –	Nil.	5.4								Dod. +	4.8			
37.	13½	orgs. –	Nil.	6.0	Ent. +	Ent. +	4.9	Dod. ++	Dod. +	(?)	Dod. +	Ent.	4.8	Dod. ++ yeasts +	Staph.	4.8
38.	20	orgs. –	Nil.	5.3	Scanty diplo.	Scanty staph.	5.0	Dod. ++	Staph. & ent.	4.9	Dod. +	Dod. +	4.8	Scanty Dod.	Nil.	6.8

1st day.					2nd day.			4th day.			9th day.			6 weeks.		
Case No.	Age in Hours.	Smear	Culture.	pH	Smear	Culture	pH	Smear	Culture	pH	Smear	Culture	pH	Smear	Culture	pH
39.	12	Orgs. —	Nil.	5.7	? orgs.	Ent. +	4.8	? orgs.	?	4.8	Mixed.	B. proteus.	5.6			
40.	24	Orgs. —	Nil.	4.9 (?)	Dod. + ent.	Ent.	4.8	Ent.	Staph. & ent.	4.7	Dod. ++	Scanty staph.	5.5 (?)			
41.	19	? orgs.	Coliform & coccus.	5.5	Dod. + ent.	Ent.	4.8	Dod. ++	?	4.7	Dod. ++	Dod.	4.8	Scanty Dod.	B. proteus.	7.6 (?)
42.	1½	orgs. —	Occas. staph.	5.9	Ent.	Ent.	4.8	Dod. ++	?	4.8	? mixed	Staph., sporing orgs.	4.9			
43.	11	orgs. —	Occas. staph.	5.4	Ent.	Ent.	4.9	Dod. & ent.	Ent.	4.8	Dod. +	Dod. +	4.8			
44.	2¾	orgs. —	Nil.	5.4	Ent. +	Ent. +	4.8	Dod. ++	Ent.	4.8	Dod. +	Dod. + ent.	4.8			
45.	8	orgs. —	Occas. staph.	5.9	Ent,	Ent.	4.9									
46.	6	orgs. —	? Dod.	5.4	Dod. ++	Ent.	4.9	Dod. ++	Ent.	4.8	Dod. ++	Dod. & ent.	4.8			
47.	16	orgs. —	Nil.	5.5	Ent. ∓	Ent. +	4.8	Dod. ++	B. proteus.	4.8	Dod. ++	B. proteus etc.	4.8			
48.	13	orgs. —	Nil.	5.7	Dod. & ent.	Ent.	4.8	Dod. +	Ent. etc.	5.2 (?)	Dod. +	Scanty staph. & ent.	4.9			
49.	7	orgs. —	Nil.	5.8	Ent. ∓	Ent.	4.8	Dod. ++	Dod. & ent.	4.7	Dod. +	Scanty staph. & ent.	4.7			
50.	12	orgs. —	Nil.	Nil	orgs. —	sporing orgs.	6.4	Dod. + ent.	Staph. & ent.	4.9	Dod. ++	Dod. ++	?			
51.	20	orgs. —	Scanty staph.	6.0	?orgs.	Ent.	6.0	Dod. +	?catarrhalis	5.8	Dod. ++	Dod. ++, ent.	4.7			

Case No.	Age in Hours.	2nd day. Smear	2nd day. Culture	2nd day. pH	3rd day. Smear	3rd day. Culture	3rd day. pH	4th day. Smear	4th day. Culture	4th day. pH	9th day. Smear	9th day. Culture	9th day. pH	3 weeks. Smear	3 weeks. Culture	3 weeks. pH
52.	26	? orgs.	Ent., staph., sporing orgs.	5.2	Ent.,scanty Dod.	Ent. & staph.	4.7	Dod. ++	Dod. ++	4.7	Dod. yeasts etc. (pus cells +)	Dod., yeasts etc.	4.9	Dod.	B. proteus.	?
53.	33	orgs. —	Staph. ent. -	5.8	Scanty Dod: catarrhalis	? Dod.	6.1	Dod. ++	Dod. +	5.0	Dod. + (leucocytes +)	Sporing orgs.	4.8	? orgs.	B. proteus (6 & 8 weeks)	7.6
54.	31	Ent. & Dod. ∓	Ent. and staph.	5.4	Scanty Dod.	Staph. & ent.	5.6	Scanty Dod.	Ent., etc.	5.6	Dod. ++	B. proteus.	4.8	Dod. & ent.	B. proteus	?
55.	35	orgs. —	Nil.	5.9	Dod. ++	B. proteus	4.8	Dod. ++	Dod. +	4.7	Dod. & ent.	B. proteus.	4.8	Dod. +	B. proteus	7.6
56.	25½	orgs. —	Scanty staph.	5.4	Dod. ++: ent. +	Ent., ?Dod.	4.9	Dod. ++	Nil.	4.8	Dod. ++	Dod. +	4.8	Scanty Dod.	B. proteus	?
57.	30	Ent. ++	Ent.	4.8	Ent. -	?Dod. B. proteus.	4.8	Dod. ++	Dod. +: cocci	4.8	Dod. ++	Dod. +	4.8			
58.	33	orgs. —	Scanty staph. & ent.	6.4	?orgs.	Scanty staph. & ent.	5.9	Scanty Dod.	Dod. +, ent.	5.8 (?)	Dod. & Ent. pus cells +	B. proteus	5.7 (?)	Debris	Nil	6.8
59.	33	? orgs.	Scanty staph.	5.8	Dod. +	Scanty staph.	5.3	Dod. ++	Dod. +	4.9	Dod. +	Staph. & ent.	4.9			
60.	37	orgs. —	Staph. & ent.	5.7	Dod. +	Staph. & ent.	4.9	Dod. +	Dod. + B. coli	4.7	Dod. - ? staph.	Ent. & Dod.	4.8	Ent. etc.	Ent.	5.6
61.	30	Gram + cocci	Scanty staph.	5.0	Dod. ++	Scanty staph.	4.8	Dod. ++	Dod. +	4.8	Dod. +	Scanty staph.	4.8	Mixed.	Scanty staph.	?
62.	44	orgs. —	Scanty staph.	5.4	Dod. ++	Dod. & ent.	5.4	Dod. ++	Scanty staph.	4.9	Mixed.	B. proteus	6.6	Scanty orgs.	B. proteus	6.8
63.	24	orgs. —	Ent. & ?Dod.	5.7	Dod. ++	Dod. ++	4.9	Dod. ++	Scanty staph.	4.8	Dod. +	B. proteus	5.0			
64.	26	orgs. —	Scanty staph.	5.4	? orgs.	Scanty staph.	5.4	Dod. ++	B. proteus.	5.0	Dod. ++	B. proteus	4.9			
65.	43	orgs. —	Scanty staph.	5.2	? orgs.	Ent.	5.4	Scanty Dod. & ent.	Ent. & staph.	5.6	Dod. ++	Scanty staph.	4.8	Mixed (scanty)	B. proteus.	5.4
66.	33	?orgs.	Scanty staph.	5.5.	Dod. +	Ent. & staph.	5.0	Dod. ++	Ent. & staph.	4.9	Dod. ++	Dod. & ent.	4.7			
67.	39	Scanty Dod.	Scanty staph.	5.2	Dod. +	Scanty staph.	4.7	Dod. ++	Staph.	4.7	Dod. ++	Ent.	4.8			
68.	38	? orgs.	Staph. albus +	5.4	Dod. ∓	Staph. +	5.3	Dod. + ⊕	Staph. +	5.0	Dod. ++	Scanty staph. & ent.	4.8	Mixed + Dod. etc., pus cells +	B. proteus	5.0
69.	28	orgs. —	Staph. +	5.4	Dod. +	Staph. scanty ent.	4.9	Dod. ++	Dod. +	4.8	Dod. ++	Staph. Dod.	4.9	Mixed +	B. proteus.	5.4
70.	40	orgs. —	Scanty staph. & ent.	5.6	Dod. ∓ ent.	Staph. & ent.	5.7	Dod. ++	Dod. + staph.	4.8	Dod. ++	B. proteus.	5.0	Mixed +	B. proteus.	5.0
71.	27½	orgs. —	Scanty staph. & ent.	5.5	Dod. ++	Scanty staph.	4.9	Dod. ++	Dod., staph. & ent.	4.7	Dod. & ent.	Dod., staph. & ent.	4.9			
72.	24	orgs. —	Scanty staph. & ent.	5.4	Orgs. —	Scanty staph. & ent.	5.4	Dod. ++	Staph.	4.8	Dod. ++	B. proteus	4.8	Dod. diph.	Scanty staph.	5.7

Case No.	Age in Hours.	2nd day. Smear.	2nd day. Culture.	2nd day. pH	3rd day. Smear.	3rd day. Culture	3rd day. pH	4th day. Smear	4th day. Culture	4th day. pH	9th day. Smear	9th day. Culture	9th day. pH	3 weeks. Smear	3 weeks. Culture	3 weeks. pH
73.	36	?Scanty Dod.	Staph. & ent. ∓	5.8	Dod. ++	Dod. & staph.	4.7	Dod. ++	Scanty staph.	4.7	Dod. ++	Occas. staph.	4.8			
74.	27	?Scanty Dod.	?	5.5	Dod. ++	Scanty staph. & ent.	4.9	Dod. ++	Staph. & ent.	4.7	Dod. ++	Scanty staph.	0	? orgs.	B. proteus.	6.8?
75.	42	Orgs. —	Scanty staph.	5.3	Orgs. —	Staph. & ent.	5.4	Dod. ++	Ent.	4.7	Dod. ++	Scanty staph.	4.8			
76.	26	Scanty Dod.	Dod. +	5.6	Scanty Dod.	Dod. +	5.6	Dod. +	? Dod.	5.4	Dod. ++	Staph. & ent.	4.9			
77.	37½	Orgs. —	Scanty Dod., staph. ent.	5.7	Dod. ++	Dod. ++	4.8	Dod. ++	Scanty staph.	4.8	Dod. +++	Occas. staph.	4.9			
78.	39½	Dod. ++	Occas. staph. alb.	4.7	Dod. ++	Occas. staph.	4.8	Dod. ++	Dod. & staph.	4.7	Dod. & ent.	Staph. & ent.	4.8			
79.	27	Orgs. —	Staph. albus.	5.4	Dod. +	Dod. & ent.	5.2	Dod. ++	Dod. & staph.	4.9	Dod. ++	Staph. & ent.	4.8			
80.	26	Orgs. —	Staph. (scanty)	5.8	Dod. & ent.	Dod. & ent.	5.0	Dod. ++	Staph. ent.	4.8	Dod. ++	Dod. & staph.	4.8			
81.	38	Orgs. —	Occas. staph.	5.7	Dod. +	Dod. & staph. ent.	4,9	Dod. ++	Dod. & staph.	4.8	Dod. ++	Nil.	4.8	Dod.	scanty staph.	6.8
82.	25½	Scanty Dod.	Occas. staph.	5.4	Dod. ++	Staph & ent.	4.9	Dod. ++	Occas. staph.	4.8	Dod. ++	Dod. ∓ staph, ent.	4.8			
83.	27	Scanty Dod.	Staph. & ent.	5.6	Dod. ++	Dod. staph. ent.	4.9	Dod. ++	Dod. staph. & ent.	4.7	Dod. ∓	Ent. ++	4.8			
84.	39	Orgs.	Scanty staph.	5.4	Dod. ++	Occas. staph.	4.8	Dod. ++	Staph. & ent.	4.8	Dod. ++	Staph. & ent. ∓	4.9	Dod. ++	Dod. +	5.0
86.	46	Scanty Dod.	Staph. & ent.	5.7	Scanty Dod.	Staph. & ent.	5.4	Dod. ++	Staph. & ent.	4.9	Dod. ++	Dod. ++, staph. & ent.	4.7			
86.	37	?occas. Dod.	Staph. albus.	5.7	Dod. +	Dod., staph & ent.	4.8	Dod. ++	Scanty staph. & ent.	4.8	Mixed flora.	Staph. & ent.	5.2	Scanty Dod. & cocci.	Staph. & ent.	6.8
87.	31	Occas. Dod.	Staph. & ent.	6.0	Dod. ++	Scanty staph, Dod. -	4.7	Dod. ++	Dod. & staph.	4.8	Dod. ++	Dod. & staph.	5.0	Scanty Dod. & ent.	Dod. & ent.	7.4
88.	38	Orgs. —	Staph.	5.8	Orgs. —	Staph. & ent.	5.7	Dod. ++	Staph. & ent.	4.8	Dod. ++	Dod. +++	4.8	Dod. +	Nil	6.9
89.	36	Orgs. —	Staph. & ent.	5.4	Scanty Dod.	Staph & ent. ++	5.0	Dod. ++	Dod. ++ E. ++	4.7	Dod. ++	Dod. ++, staph. & ent.	4.8			
90.	39	Dod. +	Scanty staph. & E.	5.5	Dod. ++	Occas. staph.	4.7	Dod. ++	Dod. ++, Staph. & ent.	4.7	Dod. ++	Dod. & staph.	4.8			
91.	36	Orgs. —	Dod. ∓	6.4	Orgs. —	Scanty staph. & ent.	6.8	Scanty orgs.	Dod. & staph. & ent.	6.8	Scanty Dod.	-	4.8			
92.	41	Scanty Dod.	Occas. staph.	5.8	Dod. ++	Occas. staph.	4.8	Dod. ++	Occas. staph.	4.7	Dod. ++	Scanty staph.	4.8			
93.	20	Orgs. —	Scanty staph. & E.	5.3	Dod. ++	Ent. and staph.	4.8	Dod. ++	Staph. and ent.	4.8	Dod. ++ pus cells +	Dod. & staph.	4.8			
94.	44	Orgs. —	Scanty staph. & E.	5.4	Dod. +	Staph. and ent.	5.0	Dod. ++	Dod., staph, col., & ent.	4.8	Dod ++	Scanty staph.	4 8			

Case No.	Age in Hours.	2nd day. Smear.	2nd day. Culture.	2nd day. pH	3rd day. Smear.	3rd day. Culture	3rd day. pH	4th day. Smear	4th day. Culture	4th day. pH	9th day. Smear	9th day. Culture	9th day. pH	3 weeks. Smear	3 weeks. Culture	3 weeks. pH
95.	36	Orgs. —	Scanty staph.	5.6	Dod. +	Staph. & ent.	5.0	Dod. ++	Dod., staph. & ent.	4.8	Dod. ++	Scanty staph.	5.4	Scanty orgs. Dod. & cocci	Staph. & ent.	7.4
96.	40	Orgs. —	Scanty staph.	5.6	Dod. ∓	Coli and ent.	5.0	Dod. ++	Coli and ent.	5.0	Dod. ++	B. proteus	4.8			
97.	29	Orgs. —	Occas. staph.	5.0	? orgs.	Dod. ∓ scanty.	5.0	Dod. ++	Dod., coli & ent.	4.8	Dod. ++	B. proteus	4.8	? orgs.	B. proteus	7.4
98.	24	Orgs. —	Scanty staph. & ent.	5.4	Dod. +	Staph., ent. & coli	5.0	Dod. +	Ent. & staph.	4.9	Dod. +	Dod. & scanty staph.	4.9	Scanty Dod.	Staph. & ent. ? Dod.	?
99.	30	Orgs. —	Staph.	5.2	Dod. ∓	Staph.	5.0	Dod. ∓	Ent. & staph.	4.9	Dod. ++	Dod. ++	5.0	? orgs.	Scanty staph.	?
00.	36	Scanty Dod.	Scanty staph.	5.6	Dod. & ent.	Staph. & ent.	5.6	Dod. ++ & ent.	Scanty staph.	4.9	Dod. ++	Dod. ++	5.2	? orgs.	B. proteus	6.8
01.	40	-	Scanty ent. and staph.	5.6	Scanty Dod.	Staph. & ent.	5.4	Dod. ++	Scanty staph. & ent.	4.8	Dod. ++	Dod. +	4.8			

APPENDIX B.

Investigation of Vaginal Secretion in Children (30 cases).

Case No.	Age (years).	Vaginal Secretion. Quantity.	Smear.	Culture	pH
1.	6	Nil	No orgs.	Scanty staph. pigmented cocci and Dod.	-
2.	5	Nil.	Scanty orgs. Most + diploc. occas. + bact.	Scanty staph. & Dod.	-
3.	4	Scanty.	? any orgs.	Staph. & ent.	?6.8 +
4.	5	Scanty.	? any orgs.	Scanty ent.	?6.8 +
5.	1½	Nil.	No orgs.	Scanty staph.	-
6.	7	Nil.	Occas. cocci & leucocytes.	B. coli & ent.	-
7.	8	Scanty.	Scanty bact. ? Dod., scanty cocci.	B. coli & ent.	?6.8 ?
8.	6	Nil.	+ cocci and ? diph; - ve bacilli	B. proteus	-
9.	7	Scanty	Orgs. scanty occas. + ve bac. (? Dod.)	B. coli	7.6
10.	5	Scanty	Occas. + cocci	B. coli	7.8
11.	2	Scanty	? any orgs.	B. proteus	7.6
12.	5	Scanty	Scanty + diplo.	Sporing orgs.	7.8
13.	5	Nil.	Scanty orgs. occas. + bac.	Coli, staph. & strep.	-
14.	7	Nil	+ cocci, - bac.	B. coli	-
15.	11	Nil	? Dod.: occas. + cocci.	Scanty coli & ent: strept	-
16.	4	Nil	? any orgs.	Scanty coli	-
17.	2½	Nil	Scanty + bac.	Dod. & coli	-

Case No.	Age (years).	Vaginal Secretion.			
		Quantity.	Smear	Culture	pH
18.	8	Scanty.	? any orgs.	Dod. & scanty coli	?7.6
19.	9	Nil	? any orgs.	Dod: ent.	-
20.	2	Nil	? any orgs.	Spreading orgs.	-
21.	5	Nil	Occas. + bac.	Staph. & Dod.	-
22.	5	Nil	Diplo c.	Staph. & prot.	-
23.	9	Scanty.	- cocci, ? Dod.	Nil.	7.8
24.	4	Little.	Diph., ? Dod.	? Dod. or diph.	7.6
25.	8	Nil	Occas. + bac.	? Diph.	-
26.	6	Little	Numerous + ve, ? - ve diph. or Dod.	Dod.	7.4
27.	7	Little	? diph. & Dod.	Dod.	7.4
28.	7	Nil	? orgs.	Nil.	-
29.	5	Nil	Numerous bact. ? Dod.	Dod.	-
30.	3½	Nil	? Dod.	Staph. & Dod.	-

APPENDIX C.

VIRGINAL LEUCORRHOEA.

Non-Infective Group.

First Examination.

Name.	Age.	Date.	Secretion.	Smear.	Culture	pH
Bell	23	21.3.30	++: viscid.	Grade A	Staph.	-
Burton	13½	21.4.32	++: viscid.	Grade A	Dod. ?ent.	4.6
Davis	23	29.8.31	do.	do.	Dod. ++	4.2
Donnelly	13	26.8.31	do.	do.	Dod. ++	4.2
Edgar	30	29.11.29	++: thick	do.	?	?
Lithgow	21	22.1.30	++: viscid	do.	Dod. & staph.	-
McClemeents	25	25.11.29	+: viscid	do.	Nil	-
McNaughton	26	3.3.30	++: viscid	do.	Staph.	-
Nolan	21	28.3.31	do.	do.	Dod. ++	4.7
Park	19	2.11.31	++ thick white	do.	Staph.	4.5
Rae	21	1.2.30	++ white profuse	do.	Scanty ?	-
Ross	18	9.7.32	++ viscid	do.	Nil.	4.5
Walters	25	10.12.29	++ yellowish	do.	Staph.	-
Whitton	24	10.12.31	++: viscid	do.	Dod. ++	4.2
Wright	31	24.10.31	+: white viscid	Grade A-B	Dod. ?	4.5
Younger	25	20.1.30	++: viscid	Grade A.	Nil.	-
Crosbie	24	20.12.31	+: viscid	do.	?	4.2
Duthie	25	4.3.32	++: viscid	Grade A-B	?	4.7
Mitchell	14	5.2.32	do.	Grade A.	Dod. +	4.4
Bridge	13	19.5.32	do.	do.	Nil.	4.6

APPENDIX C.

VIRGINAL LEUCORRHOEA.

Non-Infective Group.

Second Examination.					Third Examination.				
Date.	Secretion.	Smear.	Culture.	pH	Date.	Secretion.	Smear.	Culture.	pH
25.6.31	++: viscid	Grade A	-	4.2	8.10.31	++: viscid.	Grade A	-	4.4
6.7.32	No leuc.	do.	Dod. & staph.	4.6	14.7.32	-	-	-	4.2
24.12.31	++: viscid	do.	Dod. ++	4.2	-	-	-	-	-
2.7.32	++: viscid	do.	Dod. ++	4.4	-	-	-	-	-
7.7.32	+: viscid	Grade A	Dod. ++	4.6	-	-	-	-	-
21.10.31	++:viscid	Grade A	Dod. ++	4.6	-	-	-	-	-
29.6.32	++:	do.	Dod.++	4.2	-	-	-	-	-
7.7.32	Nil.	Grade A	-	4.2	15.2.30	-	Grade A	Dod.	?
8.1.30	-	Grade A-B.	Nil.	-	-	-	-	-	-
29.6.32	+	Grade A	Dod.	+4.4	7.7.32	+:improved	Grade A	Dod.+	4.6
30.6.32	+	do.	Nil.	4.6	-	-	-	-	-

APPENDIX

VIRGINAL

Infective

2.

LEUCORRHOEA.

Group.

Name	Age.	First Examination. Date.	Secretion.	Smear	Culture	pH	Second Examination. Date.	Secretion.	Smear.	Culture	pH	Third Examination.
Armstrong	26	30.1.30.	++: irritating, yellow	Trich. + pus + Grade C	Staph.	-						
							9.7.32	++: watery, frothy.	Grade C pus + Tr. +++	Dod. & ent. & scanty staph.	5.8	
Cuthberts	18	5.2.31.	++: watery.	Grade C	?	5.0						
Eccles	25	7.12.29	++: irritating, yellow	Grade C	Nil.	-	23.10.31	Watery, yellowish.	Grade B pus + Tr. +++	Mixed ? Dod.	5.8.	30.6.32 Watery, Grade B, Tr. ++ pH 5.6
Goate	26	12.9.30	++: yellow	Grade C pus ++ Trich. +	-	-						
Main	22	3.12.29	++: irritating yellow	Grade B-C pus +	Staph.	-	14.10.31	Watery, yellowish.	Grade C pus + Tr. +	Mixed: ent. staph. etc.	5.6	
Purdee	29	21.5.31	+++: watery	Grade C pus + Trich. +	Diph. staph. etc.	5.6	2.10.31	++	Grade C pus + Tr. +++	Mixed diph. staph. etc.	4.9	
Roseboom	23	14.2.30	+++: irritating	Grade C Trich. +	Staph.	?	14.10.31	+ watery	Grade C. pus ++ Tr. ++	Diph. & Dod.	6.0	
Smith	30	10.12.29	+	Grade C	Staph.	-	16.10.31	Watery, yellow	Grade C. pus +++ Tr. +++	Staph. diph. Tent.	-	26.11.31. Watery Grade C Tr. +++ pH 6.0
Speirs	17	10.1.30	+++	Grade C Trich. +	?	-						
							12.7.32	Scanty	Grade B-C Tr. +	Sporing org. ?Dod.	5.2	
Sloan	21	12.12.31	++: white	Grade A-B pus - + Trich. nil.	Dod. & diph.	4.6	15.10.31	+	Grade B-C pus ++ Tr. -	Diph: scanty Dod.	5.6	
							9.7.32	-	Grade A-B pus + Tr. nil	Dod., ent. & staph.	4.7	
Halcrow	26	3.3.30	++: white	Dod. ++ yeasts	Monilia	-	28.2.30	Purulent.	Yeasts & Hyphae	Yeasts +	-	30.7.30. Culture - yeasts + (Fourth examination - 15.10.31. Grade A flora: cult.-Dod.: pH 4.2.)