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Primary ovarian insufficiency previous to the climacteric : with special reference to symptomatology and diagnosis

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PRIMARY OVARIAN INSUFFICIENCY PREVIOUS TO THE CLIMACTERIC WITH SPECIAL REFERENCE TO SYMPTOMATOLOGY AND DIAGNOSIS

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I

INTRODUCTION

Dr. William Osler has said, "There is perhaps no more fascinating story in the history of science than that of the discovery of the ductless glands. The study of the interaction of these internal secretions, their influence upon development, upon mental processes, and upon disorders of metabolism, is likely to prove in the future a benefit scarely less remarkable than that which we have traced in the infectious diseases." Our knowledge of these endocrine glands has been greatly increased since this statement was made. Yet new and perplexing problems continue to arise as more and more work is being done, showing the unlimited possibilities that further investigation will unfold in regard to diagnosis and treatment of endocrine disorders. In no branch of medicine is it more essential to follow closely the newer work as regards the isolation of the products of those glands which control the physiologic balances than it is in the field which concerns the normal as well as the abnormal ovarian functions.

It is well known that the human ovary begins to function some time previous to puberty and continues to do so up

to or shortly after the menopause. The ovarian hormones have two purposes, that of influencing the development and physiology of the genital tract and that of promoting the sense of well-being of the human female. Work of the last two decades has shown that a definite syndrome of symptoms may develop from ovarian hypofunction. This hypofunction may develop in otherwise normal adult individuals having no relationship to the menopause or to surgical removal of the ovaries.

The purpose of this thesis is then, the discussion of the symptomatology and methods of rational diagnosis of primary ovarian insufficiency, limiting it to the type of insufficiency which may be found in the young adult woman previous to the climacteric who have not had surgical removal of their ovaries. Ovarian insufficiency that may result from disease processes or from other endocrine dysfunction will not be considered in this paper.

II

SYMPTOMATOLOGY OF OVARIAN INSUFFICIENCY

History of the Development of Symptomatology

In 1914, Frank (19) recognized that there was a definite syndrome associated with hypofunction of the ovaries. He said that ovarian hypofunction could be recognized anatomically; by aplasia of the genitals, systemically; by the signs of infantilism, and symtomactically by scant or infrequent menstruation, amenorrhea, dysmenorrhea, and sterility. At this time he did not describe the nervous and mental symptoms soon to be associated with insufficiency.

In 1924, Frank (20), in addition to reiterating the symptoms he had previously attributed to ovarian insufficiency, relates of advances made in the past ten years as regards normal and abnormal ovarian functions. He believes that "deficiences in follicle secretion, possibly also in other endocrine glands during the formative periods, produce fetalism and infantilism. When this deficiency first develops after puberty, hypofunction of the genital system results." Hutton (40) in the same year,

mentions the abnormal menstrual functions described by Frank, and also states that he found abdominal pain in a majority of his patients with ovarian dysfunction. This pain occasionally closely resembled that of appendicitis. Ovarian insufficiency, he feels, is responsible for the removal of some of the healthy appendices in the past, due to the fact that the surgeon was not well enough aquainted with endocrinological make-up of his patients. He is also the first to describe nervous and mental symptoms as a part of this syndrome heretofore thought to be found only at or near menopause.

In 1926, Rubin (56) thought there was a relationship between hypofunction of the ovary and sterility. In 1927, Fishbaugh (15) showed a relationship between headache and ovarian dysfunction. In 1929, Chalfant (9) emphasized the views of Frank.

However, it has been the period between 1930 and 1940 that most of the work on the syndrome of ovarian insufficiency has been done. The early part of this period showed that the ovarian syndrome was definitely correlated with the same types of symptoms as were seen in the menopause although of a less severe nature. In 1931, Werner (64) described symptoms accompanying ovarian hypofunction. Horsley (39), Hall (30), in 1933; Dunn (11), in 1934; Albright and Halsted (2); Hawkinson (32), in 1935; Schreider (59) and Fancher (12) in 1936 were the leaders in the field of the correlation of clinical symptoms and

signs with ovarian insufficiency.

SYMPTOMS

Not infrequently one may see the type of patient who has a variety of indeterminate pains. She is nervous and irritable with vague and indefinite pains and is always fatigued. She generally shows some form of maladjustment of marital or sexual life. Physical and laboratory examinations reveal no abnormalities. Therefore, one solution has been to assure her that as far as can be determined there is nothing organically wrong. This assurance may be followed by the suggestion that she is run down and tired and that perhaps a vacation and a long rest will restore her to good health once again. Or after psychoanalysis and various kinds of psychiatric examination to determine the underlying cause or precipitating factors of the patient's neurasthenia, she may be dismissed as being a hypochondriac. Then if ovarian hypofunction is present, the derangement must either adjust itself spontaneously or it becomes progressively worse. Of course all of the patients with this particular set of symptoms should not be diagnosed as having ovarian dysfunction and a great many women with these complaints may not have any basis for them from endocrinological or any other standpoint. Yet a certain percentage of these women have symptoms directly related to ovarian insufficiency and deserve as much at-

tention as merits any gynecological problem. Perhaps a great many of the women formerly classified as neurotics or neurasthenics could have been relieved of most of their symptoms, had the attending physician considered the possibility of ovarian dysfunction. For this reason, it is necessary to remember the part the ovary plays in the sense of well-being of the individual and try to determine the part that derangement of ovarian function plays in the indefinite symptoms of the patient.

The best series of cases that has been run to date is Hawkinson's group (33). In this series of 300 cases he has excluded all patients with artificial menopause or natural menopause. Also, the attempt was made to omit all patients from this series in which the symptoms might be accounted for by organic complications. The patients ranged from 14 to 35 years of age with the average age of 27 years. The duration of symptoms was 14 months. In 34 of the patients the symptoms had been present since the menarche.

Table I shows married women present two-thirds of the cases and also that abortions had occurred in over half of these married women. A definite time could be set for the onset of symptoms in 141 cases. A number of the cases were found to occur following pregnancy. Symptoms commenced after abortion and salpingectomy in 79 of the cases. The miscellaneous data presented in the 300 cases is given in Table I.

TABLE I

Miscellaneous data in 300 cases.

	Number of Cases	Percentage of Cases
Married	210	
Borne children	136	64.7
Abortions	111	52.8
Unmarried	90	
Borne children	6	6.6
Abortions	9	10.0
Syndrome began		
Following pregnancy	62	20.6
Following abortion	48	16.0
Following salpingectomy or oophorectomy	31	12.4

The syndrome accompanying the 300 cases is arranged in Table II in order of frequency of complaint.

TABLE II

Syndrome Accompanying 300 cases.

	Percentage of Patients with Complaint
Nervousness, subjective	96.0
Fatigability and lassitude	93.
Menstrual disturbances	88.3
Fatigue on awakening	79.3
Worry, trivial matters	69.
Cardiac palpitation tachycardia, etc.	67.3
Frigidity	64.6
Irritability	63.6
Excitability	52.
Depression and crying	50.3
Headaches	46.
Sleep disturbed	39.6
Vague and indefinite pains	38.3
Gastric disturbances	35.
Decreased memory and concentration	26.6
Numbness and tingling	20.3
Flushes and chills	18.

The symptoms are very similar to the menopausal syndrome, and are due primarily to deficiency of estrogen. With the exception of menstrual disturbances and changes in the vaginal smears, they are of a subjective nature. Hawkinson believes that the subjective symptoms are the result of instability of the autonomic nervous system from glandular imbalance. However, since the deficiency is less than in the menopause, the symptoms are correspondingly less severe.

A majority of patients complained of nervousness. This was a constant symptom but was usually of subjective type. The patient felt she was under great strain and tension but objectively most of the patients seemed quite calm.

Fatigability and lassitude were usual subjective symptoms. Many patients noticed fatigue on awakening in the morning after a full night's rest as well as being tired out after exertion of only a mild nature. 40% of this group showed disturbances in sleep. A few complained of disturbing dreams.

265 patients complained of definite menstrual disturbance. 174 suffered from scanty menstruation. Dysmenorrhea was present in 89 and irregular menstruation was present in a like number. Menorrhagia, metrorrhagia and secondary amenorrhea were not very common.

Trivial matters caused the patients to worry excessively. Had the patients been in good health, none of them would have given a second thought to the problems that caused

them considerable trouble and worry with this condition. A majority of these patients given to excessive worrying also showed a great mental depression accompanied with unwarranted crying spells.

Cardiac palpitation and tachycardia were the most common cardiac symptoms. No demonstrable pathology could be demonstrated to account for these findings. These symptoms were without relation to exertion, oftentimes occurring when the patient was resting or upon arising in the morning.

Frigidity occurred in over half of the patients. Many patients were able to say that the frigidity had followed the onset of their other symptoms of hypofunction. Excitability and irritability as evidenced by exaggerated response to slight emotional shocks were common findings.

Headaches were found in about half of the patients; 59% of whom suffered from menstrual migraine. The location of the pain was varied, but a group of 78 patients had definitely localized occipitocervical aching. Vague and indefinite pains occurred without definite pathology. Pelvic symptoms were not uncommon and were without demonstrable organic pathology. Because of these symptoms many of these patients had had one or more pelvic operations without relief.

Difficulty in remembering recent events and reduced powers of concentration were noticed frequently. Numbness and

tingling were not encountered as frequently as in patients at the menopause and formication was infrequent. Flushes and chills were complained of by 18% of the patients. This is much below the percentage of the same symptom present at the menopause, but it is worthy of mention that hot flushes were complained of by 18 of the patients between the ages of 14 and 21 years of age.

Hawkinson started his work on symptomatology of ovarian function in 1935 and the final results were published in 1939. The findings he has made are of great importance in the study of ovarian hypofunction because he very carefully selected his cases, omiting all cases whose symptomatology was menopausal in origin or in which symptoms were complicated by organic pathology. Thus a true determination of the actual syndrome of insufficiency may be derived from his large series of patients. The bulk of the literature on this subject has made very little attempt to separate premenopausal symptomatology from those due primarily to the menopause.

Hutton (40), in 1924, associates a great number of symptoms with ovarian insufficiency. He listed the most common symptoms as backache, nausea and vomiting, hot flashes and nervousness, mental depression and mental and physical sluggishness especially near the menstrual period. Other symptoms mentioned by him included numbness and tingling of the extremities occurring most frequently at night. A number of patients in his series complained of obstinate canker sores which became much worse at the

menstrual period. A group of patients had acne like eruptions about the face. The majority of the patients in the series lacked their usual strength and endurance. Their resistance to infection seemed to be lowered and colds and sore throats associated with their menstrual period were common findings.

Fishbaugh (15) in 1927, believed that headache is a symptom commonly found in ovarian dysfunction. He had a series of 8500 gynecological cases, out of which headache was the chief complaint in 47 cases. Of these patients, 15 had headache uncomplicated by any organic pathology that might account for the complaint. The headaches of these individuals were not characteristic and did not differ from headaches due to other causes. The type of headache in these 15 patients was associated with nervous symptoms and other features of ovarian insufficiency. Fishbaugh also noted that this group of patients show varying degrees of physical and mental depression, irritability, excitability, sleeplessness and restlessness. In some instances the nervous manifestations were almost psychopathic. Menstrual disorders, usually scanty flow of short duration, were always associated. Four of these patients during pregnancy noticed a cessation of their headache. Fishbaugh is of the opinion that these patients obtained relief from their head pain by correction of the ovarian dysfunction.

Rubin (56) in 1928, is the first to relate ovarian hypofunction with sterility. He says that in ovarian hypofunction,

the opportunity for fertilization is diminished in proportion to the reduced ovulation of the individual. With a series of 1044 cases he attempts to show that the natural incidence of fertilization is smaller in women with habitually delayed periods than in women who menstruate normally.

In 1930, Macomber (46) attempts to correlate hypofunction of the ovary with sterility. He cites the cases of several married women childless for a period of years who after ovarian hormone therapy for several months, became pregnant. All of his cases show an amenorrhea of varying degrees of severity. He believes that ovarian deficiency or lack of ovulation is caused by anything which seriously impairs ovarian circulation, or by constitutional and emotional factors which act upon the ovarian function. "In some of these later conditions, e. g. toxic goiter or diabetes, sterility may be only incidental to the primary cause but in other conditions sterility may be the only indication of disturbed function of the ovaries".

Werner (64) in 1931, and again in 1935 (65), describes a definite syndrome of symptoms that he found associated with hypoovarianism. Included in his series, however, were patients who were at or near the menopause and those who had been surgically castrated as well as those patients with pure endocrine dysfunction. According to him the syndrome may be objective or subjective. Objective symptoms are the various types of menstrual disorders, such as irregularity, hypomenorrhea, decreased duration

and amenorrhea. He finds that menorrhagia and metrorrhagia were symptoms in some patients but were found more frequently in the women who were approaching their natural menopause. Obesity of the gonadal type, characterized by the deposition of fat over the trochanters, mons veneris and in the breasts was frequent. In advanced cases he reports atrophy of the genitals, and of the glandular structure of the breasts with the loss of genital and axillary hair.

Subjective symptoms are the ones which cause the patient the discomfort and it is for these complaints that she will consult the physician. They may be classified as nervous, circulatory and general.

Table III shows the types of symptoms included under each heading.

TABLE III

Nervous	Circulatory	General
Nervousness, subjective	Hot flushes	Lassitude
Excitability	Tachycardia, palpitation	Fatigue
Irritability	Dyspnea	Constipation
Headache	Vertigo	Vague pains
Ocipitocervical pain	Scotomata	Obesity
Decreased memory and concentration	Cold hands and feet	Menstrual disorder
Depression, crying	Numbness and tingling	Amenorrhea
Psychosis	Pulse average 76/Min.	
Formication	Blood pressure Average 123/76	
Sleep disturbed	Pulse pressure Average 76	

TABLE IV

Syndrome accompanying deficiency or absence of Ovarian Hormone (follicular) in 197 cases. 53 castrates, 96 menopause, 48 involutional.

Symptoms	Percentage of patients with complaints Werner	Hawkinson
Menstrual disturbances	99.2	83.3
Nervousness, subjective	97.6	96.0
Hot flushes	89.0	18.0
Excitability	85.7	52.0
Fatigability and lassitude	83.7	93.0
Depression and crying	77.4	50.3
Constipation	76.2	35.0
Irritability	75.1	63.6
Tachycardia, palpitation and dyspnea	68.8	67.3
Vertigo	67.4	- -
Decreased memory and concentration	66.8	26.6
Sleep disturbed	66.1	39.6
Amenorrhea	57.6	- -
Headaches	56.4	46.0
Psychosis	52.2	- -
Occipitocervical aching	50.6	- -
Scotomata	49.4	- -
Numbness and tingling	48.3	20.3
Cold hands and feet	35.3	- -
Formication	25.4	- -
Vague pains in involutional melancholia	77.1	38.3

Table IV gives Werners series of 197 cases which includes 96 cases at or near menopause, 57 castrates, the remaining being ovarian insufficiency of unknown etiology. The column on the right compares the percentages of compaints that were found in Hawkinson's series of 300 patients which included only those patients with ovarian insufficiency of unkown origin. There is rather a close correlation between the two series of cases showing that the hypoovarian state of unknown origin gives rise to a syndrome close-

ly comparable to that of the menopause and surgical castration.

The first association of ovarian hypofunction with migraine was made by Blakie and Mossack (7) in 1932. They find that the disorder often makes its first appearance around the age of puberty, just at the time the ovaries are beginning to become active. They observed that many of the patients have associated menstrual abnormalities and that the migraine seemed to occur most often during the time of menses or that it occurred at some definite phase of the menstrual cycle. Their series of twenty cases was treated with an ovarian hormone which in addition to relieving the pain, also improved the menstrual disorders, caused a loss of excessive weight and promoted a sense of well-being in the patient. Thus, from a therapeutic standpoint they proved that their cases were at least in part caused by ovarian insufficiency.

Hall (30) in 1933, contributes the idea of ovarian deficiency in those individuals who were the constitutional inadequate type as first described by Alvarez. Hall believed these people do not develop genitally to maturity as does the normal individual and that they manifest their inadequacy by scanty flow and varying degrees of amenorrhea. Some have the angioneurotic symptoms most frequently found in women of the menopause. He associates menstrual migraine with ovarian dysfunction. He makes the statement that one third of the patients with insufficiency show arthritic, rheumatic, or neuralgic conditions. He states also that hypoovarianism causes an increased susceptibility to

infection, angioneurotic dyscrasias, the gonadal type of obesity and scanty menses.

Saxon (57) in 1933, agrees with Hall in that there is a definite relationship between hypofunction of the ovaries and the development of chronic arthritis. He has noticed the frequency of amenorrhea in the initial stages of the disease, especially when occurring in the earlier years of life. He finds that there are cases of spontaneous recovery from the arthritis of a deforming type during pregnancy, due to the hypersecretion of the first few months of pregnancy. This, he believes, helped to prove his contention that chronic arthritis is a part of the syndrome of hypofunction.

Support of the idea that migraine might be one of the manifestations of insufficiency is made by Riley Brickner and Kurzrok (54) in 1934. They have made hormonal studies over a prolonged period of time on the urine of 13 patients. They discovered that theelin is almost consistently absent from the patients in this series. However, the study of the daily urine excretion recorded the occasional presence of theelin in all but one of the menstruating individuals. Even when present the amount of theelin rarely exceeded 5 Rat Units per liter of urine, which is definitely below normal values. The results of their present studies suggest that migraine is a result of hypofunction of the ovary, or hypophyseal hyperfunction, in which case the ovarian insufficiency would be secondary.

Nervous and mental disorders are associated with hypofunction by Dunn (11) in 1934. He is impressed by the incidence of menstrual disorders in mental diseases and believes that menstrual disturbance causes an exacerbation of mental symptoms in some of the cases investigated. Even with a long history of ovarian insufficiency, he found that patients with nervous and mental symptoms are not obese. Their symptoms gradually increased in severity. Most of the patients showed a more or less constant headache but other symptoms of the hypofunction such as vertigo, neuritis and paresthesia were not constant but occurred irregularly having no relationship to the menstrual cycle. Restlessness and somnolence were quite common in these individuals. The patients are the lean sympathetic types, appearing to be older than their real ages.

In those nervous and mental patients with hypoovarian symptoms, headache is the most constant symptom. It has a definate relationship to the menses, occurs just before, during, or just after the period. It generally appears in the occipital region and is often associated with acute tender areas about the occipital insertion of the sternocleidomastoid muscles. The pain may migrate to the area of the temple or the vertex of the head. From here it travels to the fronto-orbital region. In 24-48 hours the other side usually becomes involved. In the more severe cases there may be complaints of visual disturbance, dimness of vision, diplopia and distortion of objects viewed.

In addition to the headache, there may be a neuritis of the upper or lower extremity with twitching of the face or eyes. Nervous irritability has occurred very frequently, and has been succeeded by melancholia, mania or hysteria. Vasomotor phenomena, including hot flashes, precordial discomfort, acroparasthesias dizziness, vertigo and sweating are occasional complaints. Nausea and vomiting are present in a few cases.

Dunn has noted that the heart rate is generally normal or slowed, the rate being influenced by the duration of the attack. The blood pressure is most often found to be quite low. Associated menstrual disorders of varying types may be noted. Hypomenorrhea and amenorrhea are most usually found; metrorrhagia rather infrequently.

The initial appearance of any of these symptoms may appear shortly before the onset of adolescence, or they may start in any decade of sex life. Dunn concludes by saying that, "the hypoovarian patient presenting nervous and mental symptoms is of the lean sympatheticotonic type. The obese hypoovarian patient seldom presents nervous symptoms, especially headache or migraine".

In 1936 further work by Glass (29) on migraine confirms the earlier findings of Riley, Brickner and Kurzrok (54). He found that he was able to increase the headache or precipitate an attack by injection of prolan. The abnormal ratio of prolan and ovarian estrogen was blamed by him as the causative factor of migraine. His series included 10 women with hypoovarian

symptoms whose outstanding complaints were periodic and paroxsymal headaches associated with menstrual disorder. The pain varied in degree and distribution. The prolan determinations were greatly increased, as high as 50 Mouse Units per 24 Hours (normal 0-25 M. U.) while the estrin values were all subnormal or absent. He concluded that the ovarian deficiency which combined with increasing hypophyseal function leads to a reversal of the normal prolan-estrogen ratio. He found that prolan therapy gave no relief neither did it intensify the symptoms. Estrogen relieved the majority of the patients, showing that estrogen deficiency is responisble in some part, at least, for migraine.

Fancher (12) in 1936, brings out the point that in severe menstrual disturbances, ovarian inactivity is more or less easily diagnosable. However, his series of cases show that there are an increasing number of women who are suffering from ovarian failure who are not aware of the fact, and whose physicians many times are also unaware of this condition. These women have the vague indefinite symptoms of excessive fatigue, nervousness, emotional instability and varying degrees of headache and backache. The average age incidence for onset of symptoms is between 25 and 35. The physician does not recognize the condition because the syndromes have not been clearly defined and also because of the difficulty encountered in getting a complete history of complaints because such patients are often reticent about giving full information unless pressed to do so.

Schreider (59) in 1936, presents a series of 85 patients with the hypoovarian syndrome. This study included a group of patients ranging in age from 9 to 91 who had symptoms of various types of menstrual disturbances. The large group of subjective symptoms were very similar in each instance regardless of the menstrual abnormality of which the patients complained. All of these symptoms were characteristic of the menopause and were found to be present in the greatest number and with the greatest severity in the patients at or near menopause. However, these same symptoms are encountered in a younger group of women in association with menstrual and reproductive abnormalities.

TABLE V

Complaint	Number of Patients
Exhaustion, tire easily	80
Irritability and nervousness	81
Emotional instability, weeping depression, suicidal tendency and disturbing dreams	69
Backache	43
Headache-migrainoid	29
Insomnia	16
Soreness of breasts	39
Leg pains	18
Nausea or vomiting	23
Cramping	31
Vaginal irritation	17
Hot flashes	18
Decrease or lack of sex desire	13
Decrease or lack of normal sex reaction	16
Associated conditions	
Acne	5
Arthritis	9
Colitis	15

Table V shows Schreider's series of 85 patients with the number of patients presenting the listed symptoms. The group includes 18 menopausal patients and 67 younger women.

The results of this series of patients closely approximates the syndromes advanced by Hawkinson and Werner given in Tables II and IV.

Table VI gives a classification of the patients encountered by Schreider in his series with the age incidence of the onset of symptoms in each type.

TABLE VI

	Number of Patients	Ages
Type I Nulliparous	19	21-36
Type II Parous	27	19-43
Type III Immediate Post Partum	5	25-34
Type IV Sterility	12	23-38
Type V Menopausal	18	30-81

Schreider believes the syndrome to be either primary or secondary. If the syndrome is primary it has been present since adolescence; if secondary, the syndrome is initiated by subsequent menstrual or reproductive events. He believes the multiplicity of symptoms due to the lack of estrogen which removes the normal inhibition of the anterior pituitary gland. This deficiency permits derangement of the function of the entire glandular system. All of Schreider's data was obtained by clinical observation. He has not made determinations of the estrogenic content of the blood or

urine nor was there any study of vaginal smears.

Randall (53) in 1937, divides primary ovarian failure into four stages. In his first stage there is an inability of the individual to produce a fertilizable ovum. These patients usually have no recognizable clinical symptoms and give entirely normal results to our known methods of investigation of ovarian deficiency but they still fail to conceive. The second stage is concerned with dysfunction of the corpus luteum. Here the patient gives the symptoms of bleeding, sterility and abortion. In the third stage of insufficiency, the function of the follicle has become sufficiently low to cause a change in the rhythmical rise and fall of the level of the estrogenic hormone of the body. It is in this phase that symptoms commonly associated only with the menopause appear. The last stage of ovarian failure is characterized by atrophy of the epithelium of the labia and vaginal wall. There is also atrophy of the internal genitalia and the breasts. Kraurosis and senile vaginitis are seen in young patients in varying degrees of severity.

Randall believes that ovarian failure may exist from the onset of puberty with mild or severe intensity. The duration of the deficiency may vary considerably. He has noticed that spontaneous remissions of the condition are seen not uncommonly and episodes of apparently normal function often are followed by recurrence of the deficiency.

Fluhmann (18) in 1938, thinks that dysmenorrhea might

be a possible symptom of ovarian insufficiency. He ran 35 tests for estrogenic substances in the blood of 19 patients. However, he discovered that the existence of a hypoplastic uterus as evidence of hypoovarianism is demonstrable in only a few patients with dysmenorrhea. Furthermore, the examination of dysmenorrheic women has not showed a regular appreciable diminution of the estrogenic hormone. He concludes that hypoovarianism is probably not responsible for dysmenorrhea. Therefore, dysmenorrhea cannot be considered as a positive sign of ovarian failure.

Finkler and Friedman (14) in 1938, believe that ovarian failure results in a patient who presents eunuchoid contours and measurements, the lateral span being three to four inches greater than the body height. Occasionally, the patient may be of normal stature, with markedly thickened lower extremities and deposits of trochanteric fat. Clinical manifestation of ovarian hypofunction may be seen in functional uterine bleeding and amenorrhea. The insufficiency may be primarily ovarian or secondary to a pituitary disturbance. These men also believe that migraine is a part of the syndrome of hypoovarianism.

Hill (38) in 1939, brings out a new symptom of hypoovarianism. He has found a hypochromic anemia in about 90% of the patients exhibiting clinical manifestations of ovarian failure. The anemia was found in varying degrees of severity. He found that pregnancy may lead to this same syndrome. 40% of his cases showed a decrease in the hemoglobin concentration and of the red

blood cells. This coupled with a definite syndrome of nervousness, fatigue, irritability and vague aches and pains is indicative of a slow and insidious failure of the glands of the endocrine system. He maintains that the endocrine system is related in some specific manner to the maintainence and stabilization of the hemoglobin concentration and the erythrocyte count of the blood. He found that the maintenance of the blood and red blood cells in the patient with insufficiency is accomplished much more satisfactorily after replacement of the ovarian hormone. He feels that a blood count is very necessary to show one of the manifestations of possible endocrine dysfunction.

Hawkinson's observations from his series of 300 cases reported (17) in 1939 bears out Hill's contention that ovarian dysfunction and hypochromic anemia are often related conditions. He found that menstrual disturbances were usually associated with hypochromic anemia. The fact that this anemia is peculiar to the female suggests some relationship between the hematopoietic and endocrine systems.

CONCLUSIONS

1. Ovarian secretions not only influence development and physiology of the genital tract but have a profound bearing upon the sense of well being of the human female. Lack of ovarian hormone may be the cause of definite symptoms.

2. The symptomatology of ovarian insufficiency may be divided into three groups; nervous, circulatory, and general.

The nervous symptoms include subjective nervousness, excitability, irritability, headache, migraine, ocipitocervical pain. decreased memory and concentration, depression, crying, psychosis, formication, sleep disturbed, worry over trivial matters, and nausea and vomiting.

The circulatory symptoms include tachycardia, palpitation dyspnea, vertigo, scotomata, cold hands and feet, numbness and tingling, pulse average per minute 76, blood pressure average 123/76, and pulse pressure average 47.

The general symptoms include lassitude, fatigue, constipation, colitis, vague pains, obesity, menstrual disorder as amenorrhea, oligomenorrhea, fatigue on awakening, gastric disturbances, acne, arthritis, decrease of sex desire, and decrease of normal sex reaction.

3. The symptoms of ovarian insufficiency are very similar to those of the menopause, from which they differ only in intensity.

4. The symptoms of many of the patients formerly classified as neurotics and neruasthenics will be found to belong under the heading of ovarian insufficiency.

III

DIAGNOSIS OF OVARIAN INSUFFICIENCY

HISTORY OF THE DEVELOPMENT OF METHODS OF DIAGNOSIS

The work of Stockard and Papanicolaou (63) in 1917, was the first step made toward a method of rational diagnosis of ovarian dysfunction. These men studied the typical estrous cycle in the guinea pig by means of examination of the histological changes of the vaginal smear. Their description of the technique of the vaginal smear examination interested many men in the years to follow, because once a normal was established, smears could be used as an indication of abnormal function of the ovaries.

Allen's description (3) in 1922, of the estrous cycle of the mouse continued the work of Stockard and Papanicolaou. In 1923, Allen(4) was able to show the menstrual cycle of the Macacus Rhesus monkey with the aid of vaginal smears.

Allen and Doisy (5) reported in 1924, that sexually mature conditions in immature female rats could be produced by follicular hormone injections. They followed the effect of the hormone by vaginal smear examination.

Frank and Meyerts (22) in 1925, provided a method of extracting the female sex hormone in the circulating blood. The

product could be injected into castrated rats and by vaginal smear be shown to produce estrus.

Hancher and Rogers (31) in 1925, believed that hypo-ovarianism, as a definite clinical entity, could be diagnosed by symptoms and laboratory findings.

Frank and Goldberger (23)(24) in 1926, determined the amount of hormone in the circulating blood and its variability during the menstrual period. They correlated different disturbances of menstruation with the rise and fall of the female sex hormone in the blood.

Rowe (55) in 1927, introduced a new idea in diagnosis of endocrine dysfunction. He thought that sugar tolerance of the individual would help in establishing diagnosis dysfunction. He found ovarian hypofunction to lower sugar tolerance in all cases.

Using vaginal smears for assays of ovarian hormone was advanced by Kahnt, and Doisy (42) in 1928. They injected albino rats with ovarian hormone to determine the potency of their preparations.

Frank and Goldberger (25) perfected the test that bears their name in 1928. The female sex hormone was isolated from the blood stream and a series of graded responses, as to the quantity of hormone present, were determined by vaginal smears of castrated mice.

Hutton (41) in 1929, emphasized the differential diagnosis of ovarian insufficiency in relation to neurasthenia and manic de-

pressive states.

Work by Kurzrok and Ratner (43)(44) during 1932 gave a method of isolating the follicular hormone from the urine. They believed that the estimation of estrin in the urine was an index for the therapy of menstrual dysfunctions.

In 1933, Papanicolaou (49), followed up his earlier work by demonstrating with vaginal smears the sexual cycle in the human female.

Fluhmann (16) in 1934, developed a new procedure for the demonstration of estrin in the blood of women. The test uses blood serum injected into spayed female mice. Microscopic examinations of vaginal sections are made to determine the amount of estrin present in the blood.

A new method of diagnosis of ovarian dysfunction was advanced by Anspack and Hoffman (6) in 1934. They believed that the endometrium of the uterus reflected abnormal functioning of the ovary. They also described the best method of obtaining biopsies from the uterus for examination. Biopsies of the endrometrium were advanced as a method of diagnosis by Randall (52) in 1935. He, however, disagreed with the previous men on the manner in which the biopsy was to be taken. In the same year, Herrell and Broders (37) reported on the various findings to be expected in an endometrial biopsy during the various phases of the menstrual cycle. They stated that Westphalen in 1936 was the first man to do endometrial biopsy. Later contributions in the field of endometrial biopsy

were made by Hitschmann and Adler in 1908, by Schroeder in 1915 and Novak (37) in 1924.

In 1935, Davis and Hartman (10) made further studies on the changes in the vaginal epithelium during pregnancy in relation to the vaginal cycle. They found that the hormones produced during pregnancy changed the histology of the vaginal epithelium. During this year Albright, Halsted and Cloney (1) discussed the means of differentiating hypoovarianism from other disease entities. They emphasized the difficulties that one encounters in a study of methods of diagnosis of endocrine disorder, especially as may be found in ovarian hormone studies. There were improvements in the Frank Goldberger test made in 1935 by the authors (36).

Papanicolaou and Shorr published work concerning their use of the vaginal smear to indicate the action to the ovarian hormone in ovarian insufficiency. This was a direct out come of Papanicolaou's work in 1917 (63). In 1936 (51), these same men published a report of the action of the ovarian follicular hormone in the menopause as indicated by vaginal smears. There was a close correlation between the vaginal smears of the hypoovarian state previous to menopause and those of the menopausal state.

In 1936, Fluhmann (17), elaborated on his method of extracting female sex hormone from the blood stream. He gives a detailed microscopic report of vaginal changes that may be expected in varying degrees of hypoovarianism.

In 1937, Burch, McClellan, Johnson and Ellison (8) dis-

cussed methods of diagnosis and made an attempt to classify menstrual disorders as they were related to ovarian dysfunction.

McCullagh (47) in 1938 believed diagnosis of ovarian hypofunction must include endometrial biopsies with an estimation of the hormone present in blood and urine. In this year Shute (61) developed a new method for detecting the estrogenic substances in the blood serum. The estrogenic content of the blood is determined by means of an estimation of the antiproteolytic power of the blood serum.

Considerable work was done in 1938 toward clarifying diagnosis of ovarian disorders and attempts were made to estimate the comparative value of the different methods of diagnosis of hypofunction. Sibernagel and Fidler (58), Finkler and Friedman (13), and Herrell (34) (35) were important contributors in this field.

Geist and Salmon (28) were among the important contributors in 1939. They attempted to evaluate the human vaginal smear in relationship to the histology of the vaginal mucosa. The basis of their work was derived from that of Papanicolaou in 1917 (63).

The most recent work on diagnosis has been done by Shute (62) and Livingston and Birnberg (45). They have summarized the methods of diagnosis now available and discuss most practical laboratory tests for ovarian deficiency. Shute believes that his rapid test for determination of estrogen in the blood stream will be of practical advantage to every physician. All of the laboratory methods of diagnosis have been greatly simplified and ren-

dered more practical for general use.

HISTORY AND PHYSICAL EXAMINATION

The diagnosis of ovarian dysfunction up to the present decade was rarely made due to lack of understanding of the subject by the physician. Had he had more definite points on which to base his diagnosis, had he had specific laboratory examinations to combine with history and physical examination of the patient, many of the cases of ovarian insufficiency could have been diagnosed. The work of Stockard and Papanicolaou (63) in 1917 was to furnish the impetus for new methods of study of ovarian dysfunction. Their demonstration of a vaginal change in histology during the estrous cycle of a guinea pig was the basis of most of the future work in the estimation of the female sex hormone present in the blood of the patient with abnormally functioning ovaries. Their work then, was the start of the laboratory methods of diagnosis of ovarian dysfunction. However, the history and physical examination of the patient is still a most important part in the diagnosis of ovarian insufficiency, and this part of the diagnosis must be as carefully performed as in any other disease complex.

As early as 1924, Hutton (40) emphasized the importance of a careful history in making a diagnosis of ovarian insufficiency. The history should include a careful inquiry into the patient's early menstrual history--the age of onset, the regularity of the

periods, the duration of flow, the presence or absence of pain, which if present, must be localized, whether in the head, abdomen, back or legs. The time of occurrence of the pain is important, whether before, during, or after the menstrual flow. It is necessary to know whether the pain is relieved or made worse by the appearance of the menses.

The history of early operation or previous infections should be obtained. A considerable number of times the patient will date their complaints from an operation. Even though it did not ostensibly involve the ovaries, it may have in some way damaged their blood or nerve supply to the extent of causing functional impairment. Infections in or about the pelvis may do the same thing. Significant points according to Hutton include a late puberty, around 16 to 18 years; onset of symptoms following an operation or an infection; and the relief of symptoms coincident with the appearance of the flow. The important things to ellicit in the history are:

1. The symptoms of the condition
2. An etiological factor
3. A connection between the periods and the symptoms

Physical findings were largely negative in Hutton's experience. When there is any change in physical make-up, the insufficiency had probably begun before puberty where it influence could be shown on the development of the bony frame work of the body. Occasionally trochanter obesity was seen in these patients but oftener it was not.

Hutton stressed the fact that a differential diagnosis is most important before any treatment is instituted. He says, "one would hardly expect to relieve a dysmenorrhea due to some malformation or malposition of the uterus by giving the patient ovarian preparations, nor would he expect to relieve a neurosis or psychosis unless it had ovarian origin".

In 1925, Hanchett and Rogers (31) published work in which they showed that disorders of the menstrual phenomenon were of diagnostic importance in determining hypoovarianism. They thought that amenorrhea and dysmenorrhea were definite indications of ovarian dysfunction. The most definite and reliable was amenorrhea and for diagnostic purposes, it need not be complete. However, amenorrhea is not always due to hypoovarianism but may be secondary to some systemic failure which is more commonly traceable to hypothyroidism than to any other cause. Only after all other origins are excluded, and this may be possible only after thyroid therapy, may the diagnosis of hypoovarianism be made. These authors believe that underactivity of the entire gland should not cause excessive flow and that amenorrhea is a definite outcome of hypoovarianism. Menorrhagia or metrorrhagia is not an indication of ovarian deficiency when they occur before the menopause.

In 1929, Hutton (41) emphasized the importance of differential diagnosis in ovarian insufficiency. It is most important to recognize tuberculosis, pernicious anemia and any other wasting

diseases, because these diseases may cause an ovarian deficiency. This deficiency should be recognized as secondary and removal of the primary cause will clear up the symptomatology of the ovarian deficiency. Neurasthenia and manic depressive states offer a great deal of difficulty in diagnosis. Many times ovarian insufficiency will cause these conditions and it may be difficult or impossible to tell how much the ovarian condition is responsible for the symptoms except by carefully regulated laboratory procedures and therapeutic test.

When the search has been narrowed to the endocrinopathies, signs of tumor of the adrenal cortex should be kept in mind, e. g. roughening of the skin, increased hair over the body, with a falling of the hair on the head or even baldness. Also, an increase in muscular strength and a general decrease in the feminine characteristics may be noted.

Hypopituitary conditions are often confused. However, there is an extreme irregularity of the periods, menorrhagia alternating with considerable periods of amenorrhea. Abnormal thyroid functions are manifest when the basal metabolism is checked and from other signs of disturbance of the autonomic nervous system.

Albright, Halsted and Cloney in 1935 (1) brought out the fact that the problem in regard to the ovary has certain difficulties which a similar problem with regard to the thyroid, for instance, does not possess. The function of the ovary is cyclical.

Normal values willhave to include levels for each of the twenty-eight days. These levels differ markedly with age. The ovary produces two hormones which are brought into existence by the anterior pituitary. Thus a careful history and physical examination is very necessary, as well as laboratory work in unraveling this knotty problem.

Sibernagel and Fidler (58) believed that a general physical examination supplemented by a gynecological examination is most necessary in the diagnosis of ovarian dysfunction. They believed that when there is a history of amenorrhea persisting beyond the age of 16, the diagnosis of ovarian hypofunction is the only one that may be made on history alone.

Herrell (35) in 1938 also endorsed the views of Sibernagel and Fidler. He believed that the weight and distribution of fat are of particular significance. In a gynecological examination presence or absence of cysts of the ovary may change the course of procedure. Herrell thought it very important to have a basal metabolism run on each patient. X-ray examination of the pituitary body in all of those patients with menstrual irregularity, especially in those patients with amenorrhea was recommended. This is necessary because one is aware of the fact that a tumor of the pituitary body may first announce its presence by amenorrhea, and it would scarely be justified to treat a patient for an ovarian insufficiency in this case.

In primary ovarian deficiency, or that in which the de-

ficiency is in the ovary itself, the activities of the other glands are normal. There is no disturbance of fat metabolism and the basal metabolism is normal. It is quite important to know whether the deficiency of the ovary is primary or secondary. A patient should not be treated for primary ovarian deficiency when the defect is in some other gland.

The majority of the men working in the field of diagnosis of ovarian insufficiency, agree that a general history and physical examination is most important in arriving at a diagnosis. However, they feel that it is necessary to stress laboratory work, chiefly because the symptoms may be quite vague and a physical examination may reveal no abnormalities.

LABORATORY EXAMINATION

VAGINAL SMEAR

After Papanicolaou's history making work of 1917, he published nothing in regard to ovarian insufficiency until 1933(49). In this article he explained the sexual cycle of the human female as is revealed by vaginal smears. He subdivided the vaginal epithelium into four zones; 1) an inner basal zone, 2) an outer pavement zone, 3) an intermediate or cornified zone, 4) a superficial or mucous zone. The menstrual cycle was divided into four phases and one stage; Menstrual phase from 1-7 days, copulative phase from 8-12 days, ovulative stage the 12-13 day, proliferative phase from 13-17 days, premenstrual phase 17th day up to onset of menses.

The histology of the normal vaginal smear in relation to the phase of the menstrual cycle is as follows:

Menstrual phase. There are numerous erythrocytes and the number of these cells increases up to the third day. The polymorphonuclear lymphocytes are abundant, while the mononuclear cells are more numerous in this phase than in any other. There is an increase of epithelial cells as the bleeding increases. The squamous epithelial cells are found of the superficial or intermediate type. Toward the end of menstrual bleeding there is an increase in leucocytes.

Copulative phase. This phase shows a relative leucopenia. The epithelial cells are of the intermediate or superficial type and there is an increased tendency toward cornification. The cornified cells are nucleated. The mucous secretion is increased.

Ovulative stage. There is again an increase in the number of leucocytes. The epithelial cells are of the intermediate or superficial variety. The cornification is most pronounced and most of the cells are nucleated. Outer pavement cells are seen occasionally. The mucous secretion is decreased in comparison with the previous phase. There is a marked increase in erythrocytes as evidence of ovulation.

Proliferative phase. This smear displays great variations. The smear will depend upon the extent of cornification processes and upon preservation of the cornified zone. If there is intense cornification, the cornified cells are high and the

leucocytes are scarce. If there is very little cornification, there will be numerous leucocytes.

Premenstrual phase There are numerous cells and the leucocytes are of the superficial cell type. The nuclei show an increase in size over the previous phases. The nucleated cornified cells are on a decrease, cells of the outer pavement type as well as mononuclears have become slightly more conspicuous. Erythrocytes are seen occasionally. The smears are of rich mucous consistency. There is a noticeable irregularity in the structure of the smear with a fragmentation and plasmolysis of the cells.

The vaginal fluid usually has a mucous consistency and contains a variety of desquamated cells, as well as leucocytes, lymph and red cells and bacteria. As the relative number and distribution of these elements change periodically, smears prepared from such fluid show modifications in their composition and structure. "The successive alternation of periods of sexual activity and inactivity imparts to the vaginal fluid a rhythmical sequence of typical cellular stages which can be easily recognized. These cyclic changes affect the entire genital tract, every change in the vaginal fluid is correlated with corresponding changes in other organs of the female genital system"(49).

In 1935, Papanicolaou and Shorr (50) demonstrated that they were able to diagnose ovarian insufficiency by means of the vaginal smear. They explained the normal vaginal findings as described above; showing a leucopenia and epithelial cells of the

squamous type, largely cornified with small pyknotic nuclei. They found that in the hypo varian state that there were many leucocytes and a predominance of epithelial, non cornified, squamous cells with larger nuclei. Compact cells derived from the deeper layers of the vaginal epithelium with large well preserved nuclei are also noted.

Thus, the work started by Papanicolaou and Shorr in 1917, resulted in a method of diagnosis of ovarian dysfunction. The vaginal smear method is an excellent method of diagnosis because of its simplicity. Daily observation, with a minimum of trauma and very little inconvenience to the patient make this type of diagnosis a method of choice. In addition, the smear method is very sensitive to slight changes in function of the ovaries and minor reactions can be noted, that otherwise might be missed.(51)

Allen and Doisy (5) in 1924, injected follicular hormone into immature rats and made a series of vaginal smears for varying amounts of hormone injected. This work was to provide a means for Frank and Goldberger to measure the amount of female sex hormone extracted by their method from the blood. When the normal smear for a known amount of hormone was determined, then injections of unknown amounts of hormone could be estimated. This injection method is an important part of the Frank-Goldberger test to be described later in this paper.

Geist (27) in 1930, published work along the same line as Papanicolaou. He also believed that the vaginal mucosa under-

goes a cyclical variation that keeps pace in a general way with the cycle of the ovary and uterus. He made descriptions of the cytology of the vaginal smear at different stages of the menstrual cycle that closely correspond with Papanicolaou's descriptions.

Geist and Salmon(28) in 1939 made a vaginal smear classification that is found in varying degrees of ovarian deficiency. This is divided into four groups. Group I includes the advanced ovarian deficiency. This smear shows a complete absence of squamous epithelial cells. Instead there are seen small round or oval epithelial cells with large staining nuclei, so called atrophy cells. These are the deep cells of Papanicolaou and Shorr. Leucocytes and erythrocytes are present in varying numbers. In some of the smears epithelial cells are few in number and there are a large number of leucocytes.

Group II or moderate deficiency shows a variable number of large epithelial cells which are of irregular shape. Nuclei are relatively large and there are numerous atrophy cells. This smear is differentiated from Group I and III by the association of the atrophy cells with larger epithelial cells.

Group III or slight deficiency shows a predominance of large irregular epithelial cells. Cells vary in size and shape with somewhat irregular edges and indistinct outline. They occur most often in clumps. Few atrophy cells are seen.

Group IV or relatively normal smear shows a large, flat clearly outlined squamous epithelium with small deeply staining

nuclei. The cells are larger and more clear cut with smaller nuclei than are seen in Group III. There are no atrophy cells and no leucocytes. This agrees very closely with Papanicolaou's discription.

The vaginal mucosa is divided by these authors into four groups depending upon the degree of ovarian deficiency. Group I or the most advanced deficiency shows a thin layer of epithelial cells which vary in depth from 1 to 6 cell rows. The epithelium is completely absent in some areas. Normal differentiation into layers is lost. There are no papillae and no cornification layer. Numerous areas of subepithelial round cell infiltration are noted.

Group II or moderate deficiency show a much thicker epithelial layer of from 4 to 10 cells. Differentiation into basalis and functionalis is present only in some areas and is not sharply defined. Papillae are few in number and shallow. Cornification is absent.

Group III or slight deficiency show a mucous membrane consisting of 10 to 20 layers. There is a definite differentiation into basalis and functionalis. The papillae are shallow and in some areas may be absent. Cornification is usually absent although a thin layer is occasionally present.

Group IV or normal mucous membrane is characterized by division into three well demarcated layers. The cornification zone is well defined and the papillae deep and uniformly distributed.

There was in general a correlation of the vaginal smear with the histology of the vaginal mucosa. Geist and Salmon believe that it is necessary to note, " this grouping is more or less artificial and not infrequently one can find different degrees of atrophy in the same biopsy speciman. The regressive process apparently does not occur uniformly".

The method of staining the vaginal specimens as used in this work is to place the vaginal secretion diluted with a little normal saline on a glass slide. This is dried in the air and stained with fuchsin for one minute. The excess is washed off with tap water. The fuchsin is prepared by adding 3 cc of fuchsin to 10 cc of 95 to 100% alcohol. 12 cc of the alcoholic fuchsin is added to 100 cc of distilled water to make the staining solution.

HORMONAL ASSAY OF BLOOD

It was in 1925, that Frank and Weyerts (22) began to publish a method for the determination of the quantity of female sex hormone in the blood. Their work on the female sex hormone was started as early as 1911, (20) but the measurement of the hormone in the blood stream did not receive their attention until early in 1922. No attempt was made to distinguish between corpus luteum hormone and follicular hormone. They made an alcohol benzene extract of the blood of five sows in estrus, five who were not

in estrus and one bull. These extracts were injected into castrated female rats. None of the anestrus blood or male blood gave positive results. The extract from the blood of the sows in estrus however, produced estrus in the castrated rats. They proved, then, that the female sex hormone can be recovered from the circulating blood and that the quantity in circulation is greater during estrus than during the interval period.

In 1928, Frank and Goldberger (24) published a method of demonstrating the female sex hormone in the circulating blood. In a series of later reports they announced revisions of this test. (23) (25). The test, in brief, is carried out as follows: 40 cc of venous blood are obtained from the patient. This unclotted blood is poured in 60 cc of pure, not denatured, 95 % alcohol which is evaporated to dryness. The residual is triturated finely and extracted twice with pure ether. This is again evaporated to dryness and then the ether soluble residue is emulsified in 2 cc of sterile, distilled water. The emulsion is divided into five doses and injected into a castrated mouse. The injections are distributed so that a period of ten hours is consumed by them. In 35-48 hours a vaginal smear is made. The approximate amount of female sex hormone present is determined by the Histology of the spread, a method suggested by Allen and Doisy (57). A vaginal smear obtained from the mouse in 36-48 hours is recorded as:

Negative--0-- If there is a preponderance of leucocytes in the spread.

Weak--plus 2-- If a large amount of nucleated epithelium is present but a few leucocytes persist.

Threshold--plus 3--If the smear contains an excess of epithelial cells and no leucocytes

Strong--plus 4--If smear shows only non-nucleated squamous epithelial cells.

In 1935, Frank and Goldberger (26) announced further revisions of their test for female sex hormone in the blood. In the new technique, 50 cc of blood is dehydrated in anhydrous sodium sulphate. The resulting powder is extracted twice with 200 cc of 95% alcohol. The alcoholic fractions are combined and evaporated to dryness on a water bath. The residuum is taken up in 5 cc of olive oil and injected into spayed mice. The bio assay is done as originally suggested by Allen and Doisy in 1924 (5). It was found that the curves drawn using this method for detecting the hormone, correspond in every way with those of the previous report, the only difference being in the increased delicacy of the reaction.

Fluhmann (16) in 1936, introduced a new method for the demonstration of estrin in the blood of women. He revised and published the work again in 1936 (17). 25 to 40 cc of blood are obtained from the patient. This blood is centrifugalized and the resulting clear serum is used for injection into adult female mice. The mice have been spayed six to seven days prior to the day the test is to be begun. A total of 4.5 cc of blood serum is given to each mouse and the test is completed in 48 hours. The serum is administered subcutaneously in the back and the site of each in-

jection is varied to facilitate absorption. Three injections of 3/4 cc are given daily, at 8 A.M., 12 noon, and 5 P.M., for two days. On the third morning the animal is killed and the vagina is carefully dissected free. The vagina is fixed in formalin and mounted in paraffin. Transverse sections are made at different levels and stained with hematoxylin eosin.

There are six possible reactions according to the amount of estrin present in the blood.

Reaction 0. Atrophy of the vagina. The mucosa shows two layers of low cuboid epithelium with occasional leucocytes.

Reaction 1. The vaginal mucosa shows two layers, a basal of low cuboidal epithelium and a superficial of tall cells. There are a few leucocytes present.

Reaction 2. Superficial cells are high, and begin to show stratification. There is a marked increase in leucocytes which may also be found in the lumen of the vagina. There is an increase in the amount of mucus present.

Reaction 3. Epithelium of the vaginal mucosa is composed of several layers and the cells at the surface are of the mucified variety. A characteristic feature often observed is the folding in of the mucosa, a type of festooning which is evidence of rapid growth. The mucosa is invaded by large numbers of leucocytes which are seen in the lumen along with epithelial debris.

Reaction 4. The mucosa is made up of from 5 to 10 rows of cells, the lower resembling the basal cells of squamous epithe-

lium while those at the surface are still of the tall mucified variety. Leucocytes are present only in small numbers.

Reaction 5. The vagina is lined by fully developed squamous epithelium with cornified cells at the surface. There are no leucocytes present. Since this test is completed in 48 hours a reaction given as 5 may consist of mucified cells at the surface with cornification just appearing between the surface and the lower stratified layers.

Two or three mice are used. The number of the reactions for each mouse is determined and added to that for the other mouse or mice. The sum is divided by the number of mice and interpreted in mouse units as follows:

Average reaction		
0-1.0	negative	Traces of Estrin
1.1-1.5	1 plus	3 M. U. per 100 cc.
1.6-2.4	2 plus	6 M. U. per 100 cc.
2.5-3.4	3 plus	12 M. U. per 100 cc.
3.5-4.4	4 plus	24 M. U. per 100 cc.
4.5-5.0	5 plus	over 24 M. U.

The error in this test arises from the variations that occur in the responses of individual mice and the problem of correctly interpreting the degree of change induced in the vaginal epithelium. The first difficulty is offset by employing as many animals as possible and the second by having all tests run by a single observer with considerable expreience.

Work by Fluhmann using this method of determination has shown that decrease in amounts of estrin is associated with ameno-

rrhea and other symptoms of hypoovarianism.

In 1938, yet another method of determining the estrogen present in the blood stream was presented. Shute (61), believed that the determination of the estrogenic substance in the blood serum could be made by means of an estimation of the antiproteolytic power of the serum. He noticed that during pregnancy the female developed properties antagonistic to the proteolytic or digestive activities of the parasitic fetus. He investigated the blood sera of a group of aborting women for antiproteolytic powers. Blood sera from these women exposed to trypsin under standard conditions displayed antiproteolytic properties not possessed by blood sera of most women during pregnancy. This was found to be due to the presence in these blood sera of an excess of estrogenic substance. Normally digestible serum could be rendered antiproteolytic by the addition of estrin. Shute then devised a qualitative test for estrogen by using this property of the blood.

1cc of blood serum is obtained from the patient. Two solutions, each made up of serum and 3.5 cc of a fresh phosphate buffer solution, are put into Wassermann tubes. One of the tubes is incubated for 30 minutes at 45 degrees centigrade. Then to both tubes add 1 cc of trypsin solution in glycerine and water. Both are incubated at 42 degrees centigrade. One sample is taken every ten minutes. To each sample, 5 cc of distilled water containing phenolphthalein is added. The samples aretitrated against fresh N/70 sodium hydroxide.

In normal sera digestion usually begins promptly in both heated and unheated tube. In Shute's series digestion commenced before forty minutes had elapsed in all of the normal sera tested. In sera showing abnormal resistance to proteolysis, digestion was sometimes found to begin in either one of the pair of tubes, when at the same time the other tube revealed no evidence of tryptic digestion. It appears the digestion by trypsin solution takes place according to the all or none principle.

Shute believed that the determination of hormone in the blood does not give a complete picture of what is happening in the endocrine system, nevertheless he felt that the estrogens seem to be the effector agents in so many of the ovarian disorders, that they may be considered by themselves as a satisfactory method of diagnosis based upon their assay alone. This test is of practical advantage because it is simple and quite rapid and it is not necessary to correlate assays of urinary pregnanediol, blood and urinary estrogen and urinary prolans A and B. Also the amount of blood necessary for the test is considerably less than that needed for the Frank-Goldberger test or Fluhmann's test. Shute believed that his results are as reliable as those of any other method of hormonal assay. He found that deficiency of estrogen is associated with oligomenorrhea and amenorrhea as well as with other symptoms of ovarian hypofunction.

HORMONAL ASSAY OF URINE

In 1932, Kurzrok, (44) described a method of determining the amount of follicular hormone present in the urine. A 24 hour speciman of urine is obtained. The urine is filtered and made acid to litmus. Next it is saturated with sodium chloride and extracted for 24 hours with ethyl acetate. The ethyl acetate is boiled off and the active principal is taken up in 7 cc of olive oil. This is injected by 1 cc, .5 cc and .2 cc doses into castrated female rats. Three rats are used and the test is positive for the hormone if two of the three rats go into estrus. Kurzrok, uses this method as a means of determining the type of menstrual disorder and also as an index for therapy. (43) He believes in the event that estrin is absent from a 24 hour speciman, a diagnosis of ovarian hypofunction is warrented.

Later work by Kurzrok in colaboration with Livingston and Birnberg (45) in 1930, show that when hormonal determinations are done on the urine, one of four responses may be expected: 1) prolan absent, estrone present. This is a normal finding. If found during amenorrhea it means that both pituitary and ovaries are functioning and that the amenorrhea is due to some other cause. 2) prolan absent, estrone absent. There is a lack of ovarian function. 3) estrone, absent, prolan present. Ovarian failure secondary to pituitary hyperfunction. 4) estrone present and prolan present. This condition is seen just before ovulation. It is also seen in

the early menopause.

Another method of determination of estrogen in the urine is advanced by McCullagh (47) in 1938. In his method a 24 hour specimen of urine is hydrolized with hydrochloric acid and extracted in a special estractor with benzol for hours. The extract is taken up in 30 cc of oil. Six spayed female rats are used for bio-assay. Two of the rats receive injections of .75 cc. Two more receive 1.5 cc and the remaining two receive 3.0 cc. Vaginal smears are taken at 48, 60 and 72 hours following injections. The smears are read according to a classification such as described by Fluhmann (16). The normal quantity of estrogen expected in the average female is around 50 Rat Units.

ENDOMETRIAL BIOPSY

Endometrial biopsy has been used for a period of years as an index of ovarian disorder. Westphalen, Hitschmann, Adler, Schroeder and Novak were early workers in this branch of diagnosis of ovarian dysfunction (37). In 1934 Randall (52) published a report in which he described the instrument used and the technique of biopsy of the endometrium. The instrument he used consists of a length of hollow metal tubing that is 32 centimeters in length and 3 cc in diameter. It is curved at one end to correspond to the axis of the uterine cavity. The end that enters the uterus is closed and on the concave aspect of the tube, .5 centimeters from

this closed end, is an opening. The distal edge of this opening is elevated and provided with two small toothlike projections. Before the curet is introduced into the uterus the cervix is thoroughly cleaned and the cervical canal and external os is painted with tincture of iodine. The tip of the curet is carried to the fundus of the uterine cavity. The concavity of the instrument is turned to the uterine wall and pressure is made, combined with a steady downward pull as the instrument is withdrawn. The specimen for biopsy will be found within the lumen of the curet, and it is usually a solid cord the length of the uterine cavity.

In the same year, Herrell and Broders (37) gave a description of the normal endometrium during various phases of the menstrual cycle. They classified the cyclic changes in the endometrium as follows; 1) the postmenstrual phase, 2) the interval phase, 3) the pregravid or premenstrual phase, 4) the menstruating endometrium. Herrell describes the atrophic endometrium or the endometrum resulting from ovarian insufficiency as one in which there is but a single layer of epithelium overlying a loose stroma. This is typical of a true atrophic endometrium. In this type, biopsy or vigorous curettage will secure only small amounts of endometrial tissue. Diagnosis may be made by the presence of small islands of epithelial cells which are usually situated in the loose stroma.

Herrell (34) believed that the endometrium reflected abnormal function of the ovary by a persistance of some phase of the

menstrual cycle. In the endometrium showing the persistent early proliferative phase, there may be expected to be an ovary deficient in the production of the luteal hormone and to some degree a deficiency in the follicular hormone. The endometrium will remain in a state of persistent proliferation. This stage is seen normally the first seven days of the cycle.

The persistent late proliferative phase shows ovarian deficiency due to lack of corpus luteum. This phase is an arrest of the regenerative process, and is like a normal late proliferative phase.

The persistent early differentiative phase shows a lack of corpus luteum hormone and is seen normally in the third week of the menstrual cycle. The persistent late differentiative phase is seen only when the degree of ovarian failure is small. The occurrence of a cystic endometrium is always indicitive of failure of the corpus luteum hormone and is the first single abnormal feature observed in histologic examination of the endometrium associated with a failing ovary.

Herrell (36) has proposed the term "persistent phases of the menstrual cycle" which will denote the deficiency in ovarian function which is manifested by endometrial change. He has found that the interpretations have been consistent to a degree that often it is possible to predict the type of clinical syndrome associated with a given histologic pattern.

If the biopsy is taken between the 22nd and 25th day,

following the last menses, the physician may learn whether or not the regeneration of the endometrium is comparable to that normally seen at this time. If there is a persistence of an earlier phase, one at once knows that there is a least a partial deficiency of the ovary to the degree of lack of corpus luteum and perhaps even some follicular deficiency (35).

Burch, McClellan, Johnson, Ellison (8) believed that the endometrial picture should be carefully correlated with the underlying glandular picture and the symptoms. They have divided the changes of the endometrium into three degrees. The first degree change is evidenced by irregular shredding of the endometrium and a persistent corpus luteum. The second degree of deficiency is shown by a glandular cystic hyperplasia and an aluteal endometrium. The third degree shows as atrophic aluteal endometrium. These men believed that disorders of the menstrual interval and flow are the result of an ovarian underfunction. Severity of the underfunction is indicated by the state of the endometrium.

SUGAR TOLERANCE

That sugar tolerance of the individual might have some relation to endocrine dysfunction was first advanced by Rowe (55) in 1927. He believed that hypofunction lowered the sugar tolerance in all of his cases. The fall is between the levels produced by overactivity of the thyroid and the level for dysfunction of the

posterior lobe of the pituitary.

Acting on this idea, Sibernagel and Fidler(58) in 1938 devised a glucose tolerance test as an aid in diagnosis of endocrine dysfunction. The patient has a blood sample taken while he is at basal metabolism. He then is given 50 grams of glucose in 300 cc of chilled lemonade. In thirty minutes a blood sugar and urine sugar is taken. The process is repeated so that in all there are three blood sugars and urines taken including the blood sugar taken at basal metabolism. This experiment is one which measures the rate at which the ingested glucose enters the blood and the rate at which the body either oxidizes, stores or excretes it. Degrees of insufficiency or hyperactivity are indicated by the degree of variation of the blood sugar curve from normal. The normal sugar curve shows a rise above the basal reading on the second blood sugar, the third blood sugar reading being lower than the second. The hyperpituitary curves were called ovarian insufficiency curves because it is well known that estrin from the ovary is the normal antagonist to the pituitary. This curve is characterized by an elevation that is markedly above normal for the last two of the three blood specimans. The authors advanced this test only as an aid in diagnosis. They did not believe that glucose tolerance alone will diagnose endocrine dysfunctions. However, the test is quite simple and may be done in the office. For this reason, it will prove of value when correlated with other symptoms and signs of ovarian insufficiency.

CONCLUSIONS

1. The procedure for diagnosis of ovarian hypofunction may be carried out as follows:

A. Complete history, covering the symptoms of the condition; an etiological factor: and a connection between the periods and the symptoms.

B. Complete physical examination including a gynecological examination.

C. Basal Metabolic Rate.

D. X-ray of head--Pituitary.

E. Vaginal Smears.

F. Hormonal Assays.
   On Blood
      1. Frank-Goldberger Test
      2. Fluhman Test
      3. Shute Test
   On Urine
      1. Kurzrok Test
      2. McCullagh Test

G. Endometrial Biopsy.

H. Sugar Tolerance Tests.

2. Nervous and mental states, tuberculosis, pernicious anemia, wasting diseases, and other endocrinopathies should be ruled out in making a diagnosis of ovarian insufficiency.

3. It is most important to discover whether the insufficiency is primary in the ovary or secondary to disease processes or endocrinopathies.

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