Papers and Originals

Amenorrhoea*

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Amenorrhoea and infrequency of the menses (oligomenorrhoea) have similar but innumerable causes, and the successful handling and treatment of these very common symptoms rest on determining the cause in each case. Normal menstruation depends on the presence of a functional uterus and on the smooth running of the endocrine system which controls it. Sometimes amenorrhoea is caused by absence of the uterus or by a failure of this organ to develop beyond a rudimentary stage, the endocrine system itself being completely normal. Indeed, when the Müllerian ducts fail to develop it is usual for the ovaries to be present and functional. So such a cause for primary amenorrhoea is not difficult to determine by the clinical findings of outwardly normal physical and sexual development and inability to palpate the uterus. In most cases of amenorrhoea, however, the uterus is present and capable of response, its failure to bleed cyclically being determined by some fault in its controlling hormones.

Mechanism of Uterine Bleeding

The occurrence of nearly all forms of functional bleeding from the endometrial blood-vessels results from their adequate stimulation by oestrogen and progesterone followed by deprivation or reduction of this stimulus. Menstruation is a hormone-withdrawal phenomenon and occurs cyclically as a result of degeneration of the corpus luteum. If the uterus is continuously rather than intermittently exposed to large amounts of oestrogen and progesterone, amenorrhoea lasts as long as the hormone influence is sufficient to maintain the endometrial structure. This is the basis for the amenorrhoea of pregnancy, the commonest cause of secondary amenorrhoea; in this case oestrogen and progesterone are produced in ever-increasing amounts by the placenta. It also explains why amenorrhoea can be induced by administering to a woman a continuous supply of oestrogen and progesterone in the form of any one of the new oral preparations. By such means, and given proper timing, a menstrual period can be deliberately postponed. Indeed, a woman can be made amenorrhoeic for many months, as happens in the treatment of endometriosis with progestogens. The exhibition followed by the deprivation of a hormone stimulus to see if withdrawal haemorrhage can be induced is the basis of the clinical therapeutic test for pregnancy.

Amenorrhoea also results if the uterus is never given an adequate stimulus by the ovarian hormones. This mechanism explains the amenorrhoea of childhood and of the post-climacteric woman.

Ageing of the ovary is the probable explanation of the menopause. Until the age of 45-55 years the ovaries continue to

harbour unripe follicles which are capable of stimulation by gonadotrophins. Then, for a reason unknown, and at an age particular to the individual, these primitive ova disappear. It is not that they are all "used up." The disappearance of ova at the normal age of the climacteric is a feature even of ovaries packed with dormant ova, as in the case of Sheehan's syndrome, where pituitary failure has resulted in no stimulation to the ovaries, or ripening of follicles, for many years previously (Sheehan, personal communication, 1961).

That the menopause is the result of disappearance of ova, and not of a failure in the output of gonadotrophins by the hypothalamus and pituitary, is established by the fact that at the climacteric the excretion of gonadotrophins is ordinarily higher than at any time in the life of any woman. This is because the hypothalamic-pituitary system is released from ovarian inhibition. It follows that in any case of amenorrhoea in which the basic error is a failure in ovarian function the excretion of gonadotrophins is likely to be raised beyond the normal range of 5-25 human menopausal gonadotrophin units in 24 hours.¹ If, however, amenorrhoea is primarily due to a failure at the pituitary level the excretion level of these hormones is low. In this way the assay of gonadotrophin excretion is of great value in assessing the cause, treatment, and prognosis in any particular case of amenorrhoea.

Ovarian Function

The rhythmical production of oestrogen and progesterone by the ovary during menstrual life is ordinarily associated with the cycle of follicular ripening, ovulation, corpus luteum formation, and corpus luteum degeneration. Formerly it was accepted that the hormone build-up consists firstly in the secretion of oestradiol by the granulosa cells of the ripening follicle, and secondly in the production of both oestradiol and progesterone by the corpus luteum during its active phase. But this simple concept is no longer tenable, and it now appears that any of the cells found in an ovary, granulosa or theca, luteinized or not luteinized, can under differing circumstances produce oestrogen, progesterone, and even androgens. Thanks to the work of biochemists such as Meyer (1955), Slaunwhite and Samuels (1956), Prunty (1956), Solomon *et al.* (1956), Finkelstein *et al.* (1961), Short and London (1961), Mahesh and Greenblatt (1961, 1962), it is now established that the hormones of the ovary are chemically interchangeable. The biosynthesis ordinarily results first in the production of progesterone, and this, by one of at least two possible synthetic chains, is converted into oestradiol, with androstenedione and other androgens as intermediary products (Sommerville, 1962; Goldzieher and Axelrod, 1963).

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¹ This does not always happen if ovarian function is destroyed before puberty. It would appear that rebound overactivity of the hypothalamic-pituitary system is to some extent dependent on its previous inhibition by ovarian hormones (Francis, 1964b).

So it is now necessary to recognize not merely a histological cycle in the ovary but a biochemical one. The latter is such that prior to ovulation progesterone is practically all converted to oestradiol, whereas after ovulation progesterone is partly converted to oestradiol and partly remains as it is. At all stages the ovary liberates small amounts of androgens.

Since there is no reason to question that the cyclical production of ovarian hormones is controlled by the periodic release of the gonadotrophins of hypothalamus and the pituitary, it follows that these hormones must control biosynthetic processes as well as morphological changes in the ovary.

Causes of Pathological Amenorrhoea

Granted that a woman has a potentially functional uterus, her amenorrhoea is always explainable on some aberration in the production of gonadal hormones.

Sometimes the ovary secretes oestrogens and progestogens in adequate amounts but produces them continuously rather than intermittently, as in the case of follicular or lutein-retention cysts, and of certain functional tumours, such as the granulosa-cell tumour and the thecoma. But in such conditions the amenorrhoea is usually short-lived and is overshadowed by subsequent heavy bleeding when the excessive hormone stimulus is ultimately reduced or becomes relatively inadequate to support the over-proliferated endometrium.

But most cases of amenorrhoea and oligomenorrhoea are explained by the failure of the ovary to produce oestrogen and progesterone in amounts sufficient to stimulate the endometrium, or to do so except at long intervals. Such a failure can arise under several circumstances.

Acquired Disease of the Ovary

Although ovarian function can be suppressed by removing the ovaries or by exposing them to massive doses of ionizing radiations, it is remarkable that ordinary disease processes affecting the gonad rarely interfere with its capacity to produce hormones. Thus bilateral ovarian abscesses, endometriosis, and tumours (excluding those which produce sex hormones) hardly ever cause amenorrhoea; no matter how extensive these lesions may appear there always remains some functional ovarian tissue. The only pelvic disease which commonly causes amenorrhoea is tuberculosis. This operates no matter whether it be in the ovary, tube, or uterus; usually all three are affected. And the infection of the ovary or other organs does not have to be gross for amenorrhoea or oligomenorrhoea to result. Often the pelvic organs feel, and even look, completely normal.

It is usually said that pelvic tuberculosis more often causes excessive uterine bleeding than amenorrhoea, but this is not my experience. Among 135 consecutive cases of bacteriologically proved pelvic tuberculosis reported by Francis (1964a), 33% of the women complained of amenorrhoea or oligomenorrhoea and only 16% of abnormal bleeding of any kind.

The special association of pelvic tuberculosis with suppression of ovarian function means that the investigation of any case of amenorrhoea is never complete until the endometrium has been cultured and injected into guinea-pigs to exclude the presence of tubercle bacilli. The importance of this procedure is illustrated by the following case.

A woman aged 30 complained of sterility and of amenorrhoea whose onset coincided with marriage eight years previously. Before marriage she menstruated regularly and had always been healthy. Full investigation, including diagnostic curettage, carried out previously in another hospital, had failed to reveal a cause for her symptoms, and it was concluded by the medical attendants and by the patient that the amenorrhoea had a psychological basis and was related in some way to the emotional upset attending her marriage.

Reinvestigation of this woman again showed no abnormality in the endocrine system or in the pelvic organs, and the original diagnosis was tentatively accepted until tubercle bacilli were grown from the scanty material obtained by uterine curettage. Menstruation became re-established within three months of the patient commencing treatment with antituberculous drugs.

Faulty Ovarian Development

Amenorrhoea is more often caused by developmental errors than by acquired disease of the ovary. And it is now recognized that these are nearly always associated with some fault in the sex-chromosome complement of the affected individual.

In the process of maturation an ovum or a spermatozoon may lose an X chromosome. The same may happen to certain cells during segmentation of the fertilized ovum. Or part of the X chromosome may be lost. On the other hand, when one of the products of meiosis or mitosis loses an X chromosome, another may gain it. So it is possible to have individuals whose sex-chromosome make-up is XO or XXX, and also individuals in whom different tissues have different chromosome complements—mosaics such as XO/XX and XX/XXX. The triple-X arrangement does not necessarily result in faulty gonad development and function (Close, 1963), but, when it does, affected individuals have amenorrhoea or oligomenorrhoea, low fertility, and sometimes impaired intelligence, but otherwise appear to be normal females.

The best example of a sex-chromosome defect causing amenorrhoea is Turner's syndrome (gonadal dysgenesis), in which the complement is typically XO but may be XX/XO, and in which the sexual apparatus, although remaining infantile, is otherwise of the normal female type and is capable of responding to exogenous oestrogens. The breasts are usually hypoplastic and body hair is absent or scanty. The characteristic skeletal defect is dwarfism, and, in addition, there may be a short wide neck (sometimes webbed), a low occipital hair line, cubitus valgus, bony deformities of the wrists, fingers, and toes, and sometimes cardiac abnormalities.

Women suffering from gonadal dysgenesis have primary amenorrhoea, and the association of this symptom with dwarfism always raises a suspicion of the diagnosis. The chromatin pattern in the nuclei of buccal squames is usually negative but occasionally positive. In the latter case it generally indicates the presence of mosaicism.

Primary amenorrhoea which is not simply explained by a slightly late puberty always suggests that the affected individual is likely to be suffering from a fundamental error in the sex-chromosome make-up. According to Jacobs *et al.* (1961), such errors can be demonstrated in 40% of women suffering from this complaint. Chromosome studies are also indicated in certain cases of secondary amenorrhoea and oligomenorrhoea.

When sex-chromosome aberrations are found the prognosis is inevitably poor and the amenorrhoea and sterility are usually permanent. Oestrogens can profitably be given to improve the development of the breasts and secondary sex characters (although they are not always successful) and to give the facies maturity. Nevertheless, such hormone therapy is often best withheld until physical growth has reached a maximum, since it may result in closure of the epiphyses.

Sex Reversal

Some individuals with a female phenotype who complain of primary amenorrhoea have testes instead of ovaries, or their gonadal tissue is partly testicular and partly ovarian. The latter condition is rare and is usually found in association with an XX sex-chromosome pattern. The former is more common and is mostly associated with the male (XY) sex-chromosome complement. Despite the nature of the gonads the external

genitalia can appear remarkably feminine, and in certain cases the vagina and even the uterus and tubes are present.

Such states of intersex illustrate the fact that the chromosome drive is only one factor in sex determination. Certain genes on autosomes are important and tend to have a masculinizing influence. The gonad itself also plays a part as an organizer in determining the development or atrophy of the Wolffian and Müllerian ducts. So a weak chromosomal, genetic, or gonadal influence in the foetus may result in the persistence of the Müllerian ducts despite the presence of testes, and what was intended to be a male individual is not masculinized. Femininity is to be regarded as a neuter state and masculinity represents a positive influence superimposed. Without this influence the individual appears feminine even though testes are present. The testes in such cases are nearly always undescended and weakly functional.

When amenorrhoea is a manifestation of intersex a clue to the diagnosis is often offered by accompanying evidence of virilism. Thus the phallus may be larger than normal for the female, and after the age of puberty facial hirsuties and deepening of the voice may develop. But this is not always the case, as is illustrated by the rather rare but very interesting syndrome of "feminizing testes." In this, undescended testes are found in what appears to be a woman of remarkable beauty and attraction, with well-developed breasts, a good figure, and soft smooth skin. Indeed, a typical feature of the syndrome is the almost complete absence of hair on the arms, legs, axilla, and even the pubes and vulva. The vulva is otherwise normal and the vagina is usually present, in part or whole. A rudimentary uterus may or may not be present.

In subjects exhibiting the feminizing testes syndrome the sex-chromosome make-up is XY and the sex-chromatin pattern in buccal squames is negative. Yet they behave as women, often marry, and have strong sexual feelings towards men. The condition can affect more than one member of the same family. It is said that women who transmit the genetic error can sometimes themselves show one characteristic of the syndrome—namely, the absence of body hair—despite having XX chromosomes and being fertile. I have seen one example of such an individual.

A woman aged 28 complained of infertility dating from marriage four years previously. Although the breasts were well formed by the age of 13 years, the menarche was delayed until she was 25. Menstruation then occurred irregularly at intervals of 4 to 12 weeks. No abnormality except hypoplasia of the genitalia was revealed by investigation. The most remarkable findings were the extreme beauty of the woman, her well-developed breasts and female figure, and the hairlessness of the axilla, trunk, pubes, and limbs. When the patient was seen again at the age of 40, the picture and symptoms were unchanged except that she reported complete amenorrhoea for 10 years. Her sex-chromatin pattern, as judged by oral smears, was positive, and it is presumed that the sex-chromosome make-up was XX or XXX. Since she menstruated for a time it must be assumed that ovarian tissue was present.

In the feminizing testes syndrome the gonads are nearly always undescended but not infrequently find their way into a hernial sac. Four out of every five affected individuals give a history of the repair of an inguinal hernia in infancy or childhood, and often a testis has been found in the sac. Indeed, it is reckoned that testes instead of ovaries are discovered at one out of every 120 operations for inguinal hernia carried out on young girls (Jagiello and Atwell, 1962).

It is generally assumed that the femininity which characterizes this syndrome is the result of interstitial (Leydig) cells of the testes producing oestrogens instead of androgens. The testis, like the ovary, and because of its similar biosynthetic processes, can produce any of the sex hormones. But this concept of oestrogenic testes is not proved. In two cases in which hormone assays were made removal of the gonads did not cause a reduction in the excretion of oestrogens in the urine (Jeffcoate, 1962).

Production of Androgens by the Ovary

Even though ovaries are present and well formed, their production of oestrogen and progesterone may be inhibited by or replaced by the production of androgens, with consequent amenorrhoea. Thus the development of an androgenic tumour such as the arrhenoblastoma results in an output of testosterone sufficient to inhibit the follicular cycle. Such tumours are usually small and can therefore be overlooked unless care is taken. Although they usually produce other manifestations of virilism such as facial hirsuties, acne, and voice-change, amenorrhoea can be the only symptom. Moreover, unlike the androgenic tumours of the adrenal cortex, ovarian arrhenoblastomas do not cause a significant rise in the excretion of 17-ketosteroids. This is because testosterone normally contributes only a small fraction to these excretion products, and, if the ovary secretes 5 mg. of testosterone daily instead of the normal 0.5 mg., the 17-ketosteroid excretion shifts only 2 or 3 mg. in 24 hours, which does not place it outside the normal range. Measurement of the testosterone level in the blood does reveal a difference, but facilities for such a test are available in only a few specialized laboratories.

Small androgenic tumours of the ovary may not be palpable on bimanual examination but can usually be seen on culdoscopy. One type of androgen-producing ovarian lesion which cannot be seen on culdoscopy, or even at laparotomy, is the hilar-cell tumour. This is a microscopic lesion which consists of groups or columns of hilar cells whose appearances are similar to the interstitial cells of the testes. They are thought to be identical and to be a source of testosterone production. When such cells, normally found in small numbers, undergo hyperplasia, amenorrhoea associated perhaps with a minor degree of virilism can result.

The ovary does not need to be the site of a tumour to be androgenic. If its biosynthetic processes are disturbed so as to produce blocks or disturbances in the normal chain of hormone production, it may fail to change progesterone into oestradiol and instead liberate excessive amounts of androstenedione, dehydroepiandrosterone, and even testosterone. Such biochemical errors, which may be variable in nature (Goldzieher and Axelrod, 1963), are seen in association with the polycystic ovaries that are regarded as the essential feature of the Stein-Leventhal syndrome. This syndrome, which has attracted much attention of late, is characterized by amenorrhoea or oligomenorrhoea, low fertility, hirsutism, and sometimes obesity. The large polycystic ovaries that are often present have a thick capsule which was at one time thought to prevent ovulation. Anovulation is supposed to lead to an accumulation of theca tissue in the substance of the ovary and around the retention cysts, and this tissue has hitherto been thought to be the source of the androgens. The treatment of this condition is generally accepted as being by resection of a wedge from each of the polycystic ovaries.

Established views on the nature and treatment of the Stein-Leventhal syndrome are put in doubt by critical studies of clinical, histological, and biochemical observations, and by the relevant literature (Gemzell *et al.*, 1958; Roberts and Haines, 1960; Goldzieher and Green, 1962; Goldzieher and Axelrod, 1963; Fienberg, 1963; Lajos *et al.*, 1963; Jeffcoate, 1964). Thus, although the presence and nature of the biochemical upsets in the polycystic ovary is fairly well established, similar functional errors can be found in normal-looking ovaries. Moreover, controlled histological studies of ovarian tissues all indicate that the amount of theca tissue, or any other morphological feature, is not related to their androgenic activity. Histologists cannot distinguish a cystic ovary producing too much oestrogen from one which produces too much androgen. Again, study of the literature reveals much confusion over the symptomatology of the Stein-Leventhal syndrome, various writers emphasizing one or other of the four basic complaints. There are also other variants. Thus, mild hypertension may

or may not be present. The excretion levels of gonadotrophins, oestrogens, pregnanediol, and pregnanetriol may be normal, raised, or decreased. The urinary excretion of 17-ketosteroids varies from 5 to 30 mg./24 hours. Even though the patient is amenorrhoeic her uterus may be normal in size and its endometrium can be atrophic, hyperplastic, or progestational.

It is also remarkable that despite other evidences of defeminization in this syndrome the breasts are remarkably well developed in 90% of cases and often show pseudo-pregnancy activity (Jeffcoate, 1964). Finally, it can be shown that local surgical treatment of the cystic ovaries gives no better results, as judged by a return of menstruation and fertility, than do patience and empirical measures such as weight-reduction and curettage (Jeffcoate, 1964).

According to the present evidence it seems reasonable to conclude that the Stein-Leventhal syndrome is not clearly defined and that it in any case represents a manifestation of a pluriglandular upset and not a primary ovarian disorder. The polycystic change in the ovaries is not constant, and when present is probably a reaction to changes in function of the hypothalamus, the pituitary, and the adrenal. Moreover, both the clinical syndrome and the polycystic change in the ovaries are liable to spontaneous cure. This again suggests that the basic fault is in the function of the hypothalamic-pituitary system.

If this conclusion is correct it means that resection of polycystic ovaries should not be the first line of treatment for patients suffering from this defeminizing syndrome. When they become available for clinical use, preparations of true human pituitary gonadotrophin or clomiphene may offer specific remedies (see below). Meanwhile, good results (50–60% cure of amenorrhoea and infertility) attend symptomatic treatment, such as the correction of obesity, sympathetic investigation, and prevarication.

Quiescence of Potentially Normal Ovaries

This concept of the Stein-Leventhal syndrome as a widespread disorder of the neuro-endocrine system leads to mention of the large group of cases in which the suppression of ovarian function, resulting in amenorrhoea, is caused by systemic disease or other endocrine disorders. In such circumstances the ovaries are quiescent but potentially normal.

Ovarian quiescence prior to puberty is explained by a low output of gonadotrophins by the hypothalamus and the pituitary. When amenorrhoea persists beyond the age of 16 to 18 years, and when it develops later in life, it is therefore common to assume that the cause is pituitary failure. In practice it is rare to see amenorrhoea as the result of proved disease in the pituitary or hypothalamus. Nevertheless, it is necessary to look for tumours and other lesions in the mid-brain in all cases of amenorrhoea. One difficulty is that a small and otherwise symptomless tumour of the pituitary may cause amenorrhoea several years before its presence can be detected by clinical signs or by radiological examination of the sella turcica. Occasionally amenorrhoea is associated with necrosis of the anterior lobe of the pituitary gland, the necrosis generally resulting from ischaemia associated with shock and haemorrhage sustained during childbirth (Simmonds's or Sheehan's syndrome). This should be suspected in all cases in which amenorrhoea follows a complicated abortion or labour. When the syndrome is complete the diagnosis is easy because amenorrhoea is accompanied by evidences of a failure of pituitary thyrotrophic and adrenocorticotrophic functions. But detailed investigation may be necessary to recognize the incomplete syndrome in which the functions of the anterior pituitary are only partially destroyed, and when amenorrhoea may be the only obvious effect.

Amenorrhoea is rarely associated with hyperthyroidism but is common in juvenile diabetes. Moreover, it is not always

possible to control the diabetes sufficiently well to permit a return of the menstrual function. Any disease of the adrenal cortex associated with overproduction of androgens is a potent cause of amenorrhoea. This symptom is seen in cases of adrenal cortical hyperplasia or tumour formation as part of the adrenogenital syndrome. In all these circumstances ovarian function is directly suppressed by the high level of androgen in circulation; other signs of virilism are usual accompaniments.

The body's response to any general debilitating disease is often to sacrifice first its reproductive function. This is manifested by amenorrhoea. The only general diseases which in their early stages, before wasting sets in, commonly cause amenorrhoea are lymphadenoma and pulmonary tuberculosis. The explanation of the sensitivity of the menstrual function to these conditions is unknown. Anaemia, contrary to the traditional view, never causes amenorrhoea.

Amenorrhoea is often accompanied by change in weight, either loss or gain. Wasting not due to organic disease in association with amenorrhoea almost invariably means a diagnosis of anorexia nervosa. This disorder, in minor degrees, is common. A young adult, usually alert and intelligent, ceases to eat for various reasons. Sometimes it is a habit which begins with dieting to improve the figure; sometimes there is a more basic psychological upset which prompts the individual to call attention to herself. She may be in need of sympathy, may be jealous of another member of the family, may be envious of the solicitude which a relative secures by ill-health. Amenorrhoea can be an early and resistant symptom of this syndrome. It is only in part explained by starvation, so even when the girl corrects her weight it can persist until the underlying psychological upset is corrected.

Conversely, amenorrhoea and oligomenorrhoea are often associated with obesity. Here again there may be a psychological basis which affects the hypothalamus to change both appetite and menstruation. But obesity itself does appear to depress the menstrual function, and in cases of overweight women mere reduction of weight by strict adherence to a low-calorific diet is often followed by a resumption of a regular menstrual cycle. This, as mentioned earlier, applies even when the obesity is part of the Stein-Leventhal syndrome. In this respect it is of interest that mere reduction in weight can correct certain upsets in adrenocortical function (Baird, 1963).

Changes in environment, in the type of work undertaken, and in the outlook on life commonly suppress ovarian function temporarily. Fifty per cent. of young girls living an institutional life develop a phase of amenorrhoea. Intensive study for examinations is another common aetiological factor.

Indeed, leaving aside pregnancy, the commonest underlying causes of secondary amenorrhoea are psychological and environmental. Such factors account for not less than 60% of cases. To recognize this is important, since secondary amenorrhoea that is psychogenic does not require and often does not respond to hormone therapy, but if well handled carries a good prognosis.

Management

What should be the clinical approach to a case of amenorrhoea? Fundamentally it is to determine the cause. In this respect the guiding rule is that every woman who complains of amenorrhoea is pregnant until proved to the contrary. This applies particularly to secondary amenorrhoea. When the complaint is primary amenorrhoea, cryptomenorrhoea has also to be excluded. Thereafter the clinical features of the case deserve the closest study.

The time and mode of onset of amenorrhoea, the history of previous illnesses, the presence of associated symptoms such as loss or gain in weight, hot flushes, infertility, hirsutism, acne, and mental disorders all give a lead. Is the patient taking drugs such as phenothiazine derivatives and reserpine, or has

she had shock therapy? These can disturb hypothalamic function.

Examination usually gives a clear indication of the presence of endocrine disturbances. In this respect special attention needs to be directed to the development of the breasts and other secondary sex characters, the presence and size of the uterus, and evidence of atrophy or oestrogenic stimulation of the genital tract. In psychogenic amenorrhoea the vaginal epithelium often appears to be still under the influence of oestrogens.

The clinical picture gives the guide to what may be necessary by way of more special investigations. But in all cases in which the cause is not clear, and often when it seems to be obvious, the investigations which can never be omitted are (a) radiological studies of the pituitary fossa and lungs, (b) pelvic examination under anaesthesia with measurement of the uterus, (c) bacteriological examination of endometrial debris for tuberculosis, (d) histological examination of the endometrium when sufficient material can be obtained, and (e) assays of the urinary excretion of 17-ketosteroids and gonadotrophins.

In all cases of primary amenorrhoea, and in certain cases of secondary amenorrhoea and oligomenorrhoea, there is a need to determine the sex-chromatin pattern in the nuclei of epithelial cells obtained by buccal scrapings. Also, when facilities are available, the chromosome complement should be studied by the appropriate examination of cultures of leucocytes and other tissues.

Those described above are the basic and essential investigations; the need for others, such as studies of thyroid function and glucose metabolism, and culdoscopy or laparotomy, depends on the circumstances of a particular case. But the fact remains that the rational and satisfactory treatment of amenorrhoea depends on determining its cause with reasonable certainty.

Treatment

The treatment of amenorrhoea is primarily the treatment of its cause, whatever this may be. When an organic basis is not found and the aetiology of the symptom is obscure, or thought to be psychological and environmental, empirical therapy with ovarian hormones is generally to be deprecated. The cyclical administration of oestrogens, with or without progestogens, for a few months may induce periodic uterine bleeding. This is a useful test for endometrial sensitivity, and may sometimes be enough to correct what has been only a minor and temporary derangement in the endocrine system. It is also valuable when there is a need to correct associated underdevelopment of the secondary sex organs and characters. But to continue such treatment indefinitely to maintain an artificial menstrual cycle is pointless.

For the purpose of stimulating ovaries to ovulate and to secrete hormones there is hope for the future when preparations of human pituitary gonadotrophins become available for general use. Gonadotrophic substances marketed in the past proved singularly disappointing in this respect, probably because, so far as follicle-stimulating hormone is concerned, they were obtained from animal sources. But extracts of human pituitary glands when injected daily for 10 to 14 days, and then followed by daily injections of human chorionic gonadotrophin, undoubtedly have the power to induce ovulation from a quiescent ovary (Gemzell *et al.*, 1958; Buxton and Herrmann, 1961; Gemzell, 1963; Crooke *et al.*, 1963a, 1963b, 1964; Edwards, 1964). By correct timing, and in suitable cases, pregnancies follow such treatment not infrequently, even in women who have long been amenorrhoeic. But it is impractical to expect enough material to be obtained from pituitary glands to serve more than a few women. However, similar good results are likely to be obtained with human pituitary gonadotrophins obtained from the urine of post-menopausal women, and such preparations are likely to be more widely available in the near future.

In view of this it has to be recognized that such treatment is indicated only in cases in which amenorrhoea and infertility are due to a failure of, or disturbance in, the gonadotrophic function of the hypothalamic-pituitary complex. For it to be successful the ovaries must contain ova capable of undergoing maturation. It would therefore be inappropriate, for example, in cases of gonadal dysgenesis, but might be valuable in cases of the Stein-Leventhal syndrome, or of necrosis of the anterior pituitary gland.

Not only is it necessary to be selective in choosing the cases for human gonadotrophic hormone therapy, it also has to be recognized that the treatment, unless carefully controlled, can have ill effects. Overdosage can produce massive enlargement of the ovaries, cystic change, and even severe haemorrhage from the ovary necessitating laparotomy (Edwards, 1964; Shearman, 1965). Such complications, as well as the production of multiple ovulation with consequent multiple pregnancy, can be avoided only if treatment is controlled and dosage determined by daily assays of urinary oestrogen and pregnanediol in each case.

Another substance reported to induce ovulation, and to be particularly helpful in restoring normal ovarian function in cases of the Stein-Leventhal syndrome is clomiphene (Greenblatt, 1961; Greenblatt *et al.*, 1961; Charles, 1962; Charles *et al.*, 1963; Whitelaw *et al.*, 1964). But this preparation can also have undesirable side-effects and is not yet available for general use.

The place for treatment with human pituitary and chorionic gonadotrophins has still to be determined and may prove to be small as measured against the large number of cases of amenorrhoea encountered in practice. Meanwhile the treatment of amenorrhoea is to correct any factor which may be concerned in its aetiology. Thus, if the patient is fat she should be made slim; if she is thin she should be encouraged to eat. The results of the most simple therapeutic measures, or of no treatment other than sympathetic encouragement, are often remarkable.

When primary amenorrhoea persists to the age of 20 years, and especially if a fundamental error in the sex chromosome complement is found, the prognosis is poor. Nevertheless, I have seen several women whose menarche occurred spontaneously at ages ranging from 20 to 27 years.

In the case of secondary amenorrhoea and oligomenorrhoea, the outlook is different. Causes for these symptoms are generally amenable to treatment. And whatever be the nature of the treatment given there is a 60% chance of cure in cases of secondary amenorrhoea of more than one year's duration. In most cases this cure is spontaneous, the cause of the symptoms having been some temporary psychological or environmental upset, or some self-correcting functional disturbance of the pituitary and hypothalamus.

Summary

Provided the uterus is present and capable of function, amenorrhoea is usually caused by a failure of the ovary to produce oestrogen and progesterone cyclically and in adequate amounts.

Acquired disease of the ovary only occasionally causes amenorrhoea.

Amenorrhoea due to failure of the gonad to develop or to differentiate to the female side is commonly associated with sex-chromosome anomalies.

Although an error in the biosynthesis of hormones by the ovary is a feature of the Stein-Leventhal syndrome, this is probably secondary to disturbances in the hypothalamic-pituitary system and the adrenal. It is liable to spontaneous cure, and resection of polycystic ovaries is often not the best treatment.

Amenorrhoea is rarely caused by organic disease in the hypothalamus and the pituitary, but is often associated with certain general disorders, with dietetic errors resulting in either an increase or a loss of weight, and with psychological and environmental changes.

Treatment of amenorrhoea depends on its cause, and the determination of this requires full investigation, which should generally include bacteriological examination of the endometrium to exclude tuberculosis.

The prognosis is good in cases of secondary amenorrhoea, menstruation returning in 60% of cases irrespective of treatment.

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Further Studies of Agents Isolated from Tissue Cultures Inoculated with Human Leukaemic Bone-marrow

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[WITH SPECIAL PLATE]

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The presence of a cytopathic agent in human-embryo-kidney-cell cultures inoculated with passage fluid from human leukaemic bone-marrow was reported by Negroni (1964) and by Inman *et al.* (1964). The presence of a mycoplasma (pleuropneumonia-like organisms: P.P.L.O.) in these cultures has since been demonstrated by Grist and Fallon (1964) and Girardi *et al.* (1965). The present paper is a preliminary report of attempts to characterize this mycoplasma and to determine its relationship to the original cytopathic agent.

Materials and Methods

Tissue Cultures.—Tissue cultures of primary rhesus-monkey kidney, primary human amnion, primary human embryo, continuous baby-hamster kidney (BHK21-C13 line of Macpherson and Stoker, 1962), and the "Bristol" line of HeLa cells were prepared in Glasgow by the standard methods of the

Regional Virus Laboratory, Ruchill (Grist *et al.*, 1965); inoculated cultures were maintained in Eagle's medium with 1% fowl serum in order to avoid inhibitors found in calf, horse, and sheep sera.

Mycoplasma Culture Media.—The media used were those described by Lemcke (1964) or Chanock *et al.* (1962). Where these media were used to grow mycoplasmas for immunization of rabbits, normal rabbit serum was incorporated in the medium instead of horse serum. For the study of glucose fermentation 1% of the sugar was incorporated in the solid medium together with indicator.

Haemolysis of Mammalian Erythrocytes.—This was tested by layering blood agar on to colonies on solid medium (Somerson *et al.*, 1963).

Serological Tests.—Tissue-culture-neutralization tests were carried out as previously described by Negroni except that the cultures were incubated stationary at 36° C. Complement-fixation (C.F.) and gel-diffusion (Ouchterlony) tests with rabbit antisera against standard strains of mycoplasma and against tissue cultures containing the cytopathic agent were performed as described by Lemcke (1964, 1965). C.F. tests with antiserum prepared against the mycoplasma strain CW were performed by a modification (Grist *et al.*, 1965) of the method of Bradstreet and Taylor (1962) with suspensions of antigen which

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