

CERVICAL MUCUS IN EARLY PREGNANCY

A thesis submitted by

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CERVICAL MUCUS IN EARLY PREGNANCY

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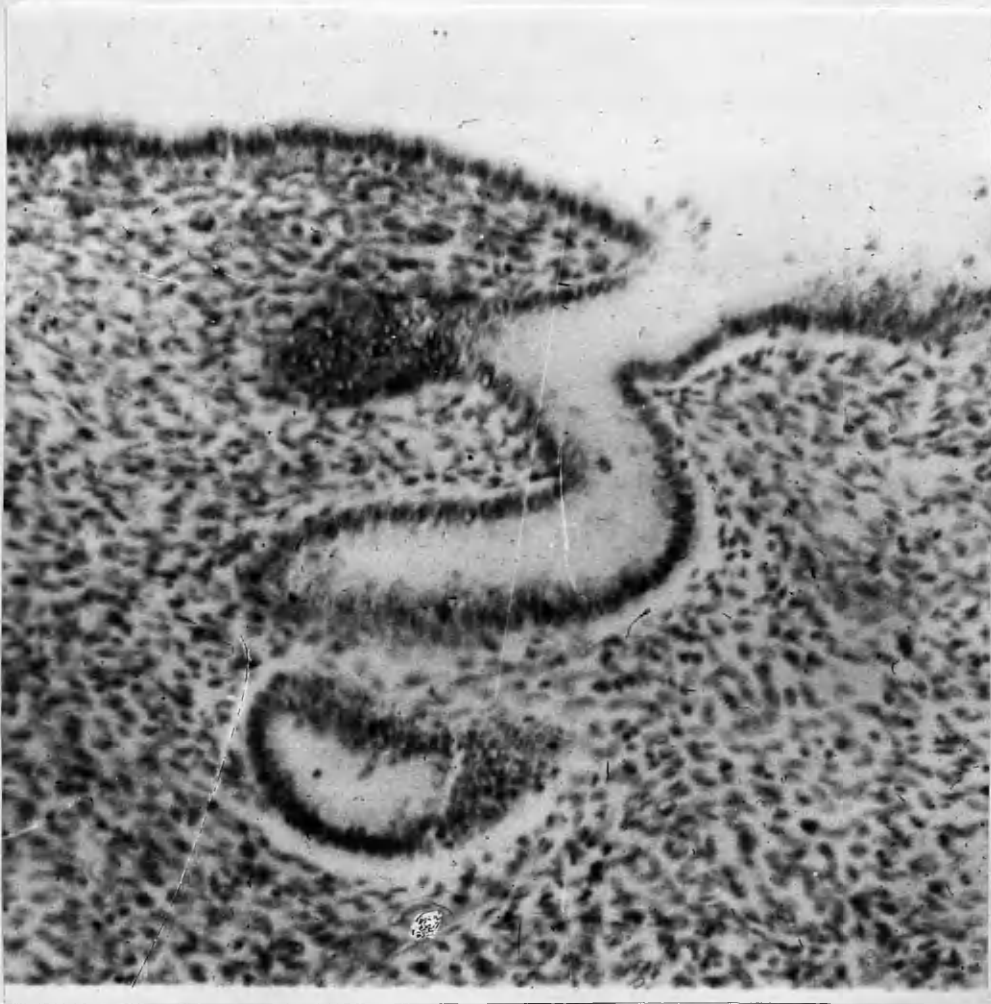


FIGURE 1.

X 250

A normal endocervical gland.

CERVICAL MUCUS IN EARLY PREGNANCY

C H A P T E R I

INTRODUCTION AND REVIEW OF THE EARLIER LITERATURE

Mucus is produced in the endocervical canal by numerous glands lined with columnar cells (Figure 1). The normal function of the mucus is to act as a barrier against infection and as a means of lubrication. Throughout the normal menstrual cycle the mucus varies in much the same way as the endometrium and the vaginal cells. Immediately after menstruation the mucus is very scanty, cloudy and sticky, but after about the 8th day it begins to increase in amount and by ovulation time, it is profuse, clear and elastic. In the second half of the cycle, it decreases in quantity, and usually about the 22nd day, it begins to become more cloudy and viscous again.

The cyclical changes in the cervical mucus were first described in 1933 by Seguy and Vimeux, and Seguy and Simonnet (1933) showed that there was a relationship between these changes and oestrogen excretion in the urine, since the amount of mucus increased in proportion to the oestrogen level. Moricard (1936) and Watson (1939) demonstrated that the administration of oestrogen increased cervical mucus excretion, reduced viscosity and improved sperm penetration. After the menopause the mucus is normally very scanty but by giving oestrogen to menopausal and post-menopausal women, Guttmacher and Shettles (1940) and Bennet (1942) caused the reappearance of mucus characteristic of reproductive life, while Abarbanel (1946) and Palmer (1946) achieved

the same result studying castrated women and were able to inhibit the mucus production by giving progesterone.

The marked quantitative variations in cervical mucus secretion throughout the cycle were measured by Viergiver and Pommerenke (1944) and correlated with basal temperature. In 1946 the same authors reported objective measurements of the variations in viscosity, and Pommerenke (1946) went on to study in greater detail the physical and chemical properties and constituents of the mucus. Their observations can be summarised as follows. The amount of mucus ranged from 20 to 60 mg. per day in the post-menstrual phase, increasing from about the 8th day to a maximum at mid-cycle of 200 to 700 mg. depending on the individual: after the ovulatory phase the quantity was reduced again to 20 to 60 mg. per day and remained at that level until menstruation. The maximum secretion preceded the rise in basal temperature by 1 to 3 days and was associated with minimum viscosity and maximum penetrability by spermatozoa. Viscosity was up to 50 times greater in the pre-menstrual and post-menstrual phases, while sperm penetration reduced from 3 mm. or more per minute at mid-cycle to 0.25 mm. or less per minute before and after menstruation. The water content ranged from 92% to 94% in the pre and post-menstrual phases, when the mucus contained relatively numerous endocervical cells and leucocytes, to 97% or 98% at mid-cycle when the mucus was relatively acellular and showed minimum turbidity and a lower concentration of carbohydrate and amino acids. The pH varied from 9 to 9.6 although it was not clear at what stage in the cycle the mucus was most alkaline.

Clift (1945) added observations on the rheological properties of the mucus confirming minimum viscosity at mid-cycle, and Cohen Stein, and Kay (1952) gave a good description of mucus elasticity or Spinnbarkeit, the term used to denote the way in which the mucus could be drawn into a thread which increased in length as the viscosity reduced, and was maximal about the time of ovulation.

There emerged from all these investigations a clear picture of cervical mucus secretion, varying throughout the cycle in amount, viscosity, elasticity, turbidity, pH, cellular content, water content, carbohydrate and amino acid concentration and resistance to spermatozoa. The secretion of mucus was clearly dependent on oestrogen production and inhibited by progesterone.

While studying vaginal smears Papanicolaou (1946) made an important observation when he noticed that cervical mucus spread on a slide and left to dry formed a pattern which he thought represented a kind of crystallization. He showed that this pattern was most pronounced near the peak of follicular activity and about ovulation time, whilst at other stages of the cycle, in pregnancy, amenorrhoea or after the menopause, there was reduction or absence of crystallization. Rydberg (1948) described the crystallization as resembling fern or palm leaves, while one of his colleagues analysed the crystals and found them to consist of 97% sodium chloride and 0.77% organic matter.

This ferning was studied throughout the cycle by Campos da Paz (1951) who showed an inhibitory effect on the crystals as on the mucus itself by progesterone. Crystallization appeared a few days

after menstruation, reached a maximum about mid-cycle, then disappeared within a few days and remained absent until the beginning of the next cycle.

Subsequently Zondek (1954) made a series of careful and valuable observations, including a detailed study of the mechanism of ferning or arborization. He noticed that crystallization and elasticity could occur independently. Experiments in vitro revealed that crystallization could be produced by a weak solution of any protein or carbohydrate present in the mucus with certain electrolytes. The proteins included albumin, globulin, and fibrinogen; the carbohydrates included glucose, maltose and glycogen; and there were two amino acids glutamic acid and cystein hydrochloric acid. The presence of electrolyte was decisive and of many salts tested only sodium chloride, potassium chloride and potassium bromide produced a reaction. Similar crystallization could be produced by making dried smears of all the mucous secretions of the body and many body fluids such as tears and cerebrospinal fluid. If the concentration of protein was too high (4% to 6%) as in colostrum, milk or serum, crystallization did not appear. In cervical mucus the crystals were inhibited mechanically by semen and blood until fresh saline was added.

Zondek also found that 1 mg. of oestrone or 0.3 to 0.4 mg. of oestradiol was the minimum amount of oestrogenic hormone capable of inducing the production of cervical mucus which would crystallise in the dried state 72 to 120 hours after a single injection, and this was

defined as one Cervical Mucus Unit. Progesterone inhibited crystallization and 15 mg. of progesterone counteracted one Cervical Mucus Unit. This minimum quantity of oestrogen did not produce any functional effect on the endometrium or the vaginal epithelium and so the cervical glands were shown to be the most sensitive reactor to oestrogenic hormone. It was suggested therefore, that the ferning response of the cervical mucus could be used for the titration of oestrogens. Zondek's final important observation was that, although all mucous secretions of the body produced some form of ferning in the dried smear, only in the cervix was the quantity and appearance of the mucus, and the presence of ferning, dependant on oestrogen and progesterone.

C H A P T E R II

CERVICAL MUCUS IN THE NORMAL MENSTRUAL CYCLEIntroduction

In 1956, after seeing a paper by Campos de Paz, the writer was struck by the simplicity of examining cervical mucus and its significance as an indication of oestrogenic activity. It seemed likely that the mucus could be used for simple clinical tests, and it was decided initially to confirm this work and study mucous crystallization by taking samples of mucus at varying stages throughout the cycle.

Method

The cervix was exposed with a speculum in a good light and the ecto-cervix gently wiped with sterile gauze to carry away vaginal debris and old mucus. A wisp of cotton wool on the end of an orange stick was introduced into the lower end of the cervical canal and rotated in order to pick up a little fresh mucus which was then spread in a thick smear on a clean, dry, glass slide and left in the air to dry at room temperature. Two smears were taken from each patient since it was quickly found that a second smear drawn from deeper mucus frequently gave a slightly different picture. Where the two smears were not identical the second one was preferred for diagnosis. A thickish smear was necessary since crystals might fail to appear if the mucus was spread too thinly.

This method of obtaining the mucus was thought to be better than the one suggested by Zondek (1954) where vaginal pipettes were used



FIGURE 2.

X 90

Salt Crystals in Cervical Mucus

Typical crystallization showing marked ferning.

Normal mucus about the time of ovulation.

to aspirate the mucus from the cervical canal and then blow it out on to the slide. The advantages of the cotton wool applicator are as follows:-

- i) A fresh sterile applicator can be used for each sample of mucus taken and so there is no risk of introducing infection or in carrying organisms from one patient to another.
- ii) The dry applicator prevents contamination with salt: the pipettes unless boiled in distilled water and dried carefully could carry minute traces of salt, which would give false positive crystallization.
- iii) The mucus is more easily transferred by the cotton wool from the cervix to the slide, particularly when it is viscous.

The smears dried in 10 to 30 minutes and were then examined under the low power microscope and graded as showing marked, moderate, or scanty crystallization (Figures 2 & 4). After a few hours the smears occasionally began to absorb moisture from the atmosphere and the crystalline picture to become blurred. Several methods were tried to preserve the smears but all stains and fixatives simply dissolved the crystals. If a dry cover slip were placed over the smear and the edges sealed with a very small amount of Canada Balsam, the crystals could be maintained virtually unchanged for several weeks. This was helpful for demonstration purposes, but in practice it was found most useful to examine the slides within an hour and record a diagnosis at

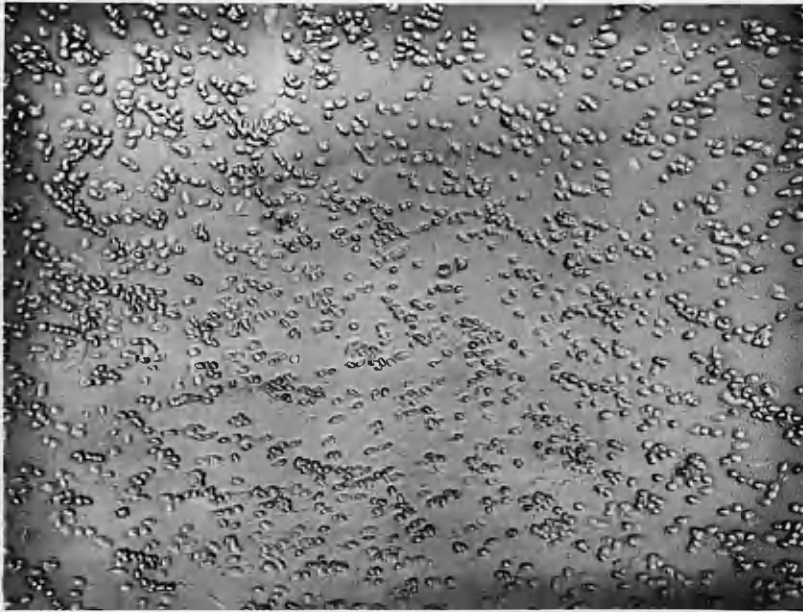


FIGURE 3.

X 90

Endocervical cells and no crystals.

Normal premenstrual or pregnancy mucus.

once, checking the slide again 12 to 24 hours later. Where a permanent record of an important slide was required, the best method proved to be a photomicrograph taken as soon as possible and certainly within 24 hours.

After a little practice it became quite a simple matter to obtain the mucus and make the slides. Disturbance for the patients was minimal and interpretation of the results was quick and easy.

Investigation

Samples of mucus were taken from 15 Outpatients initially. They had been referred to the Gynaecological Clinic and were chosen for testing because it was expected that the mucus would be normal in each case. The patients were distributed in such a way that mucus smears were obtained roughly every second day throughout the cycle giving a complete normal range.

A second series was taken from 21 patients who had been admitted to the hospital and were due for curettage the same day or the next day. A mucous sample was taken in each case and the appearance of the dried smear was related to the histology of the endometrium obtained at curettage. Eight of these samples were premenstrual and the remaining 13 were scattered evenly throughout the cycle.

In order to determine the normal appearance after menstrual life, mucous samples were also taken from 10 post-menopausal women. In addition, a few smears were obtained from patients during menstrual life who were actually bleeding at the time, and these are reported separately. Finally, some of the crystalline material accumulated



FIGURE 4.

X 100

Atypical crystals showing moderate to scanty ferning.

Normally seen before and after ovulation.

from several slides was examined by X-ray diffraction to find out its exact composition.

Results - Normal Smears

The first 15 smears taken from the Outpatients gave a good picture of the range throughout the normal menstrual cycle. It was confirmed that immediately after menstruation mucus was scanty, cloudy and very viscous, but about the 5th to the 8th day it began to increase in amount, become clearer and gradually increase in elasticity. About the same time fragments of fern-like crystals appeared in the dried smears and as the amount of mucus increased and its viscosity reduced, the density of the ferning increased with a maximum at mid-cycle. Figure 2 shows the normal appearance of the dried smear about the 14th day with strong typical crystals and no cells. From mid-cycle onwards the amount of mucus gradually decreased and the typical fern crystals reduced again to smaller particles. About the 20th to the 22nd day as the mucus became cloudy and viscous again the ferning disappeared completely. Figure 3 shows the normal pre-menstrual smear with endocervical cells only and no crystals.

In the phases from the 8th to the 12th days, and the 16th to the 22nd days, ferning crystals could be recognised on the smears but in a much less definite form, as can be seen from Figure 4 which shows a full range of partial or atypical crystals from quite obvious ferns down to fine branching threads and eventually minute crystalline dots.

It was truly fascinating to take a smear of clear elastic mucus about mid-cycle and watch it under the microscope while it dried. At

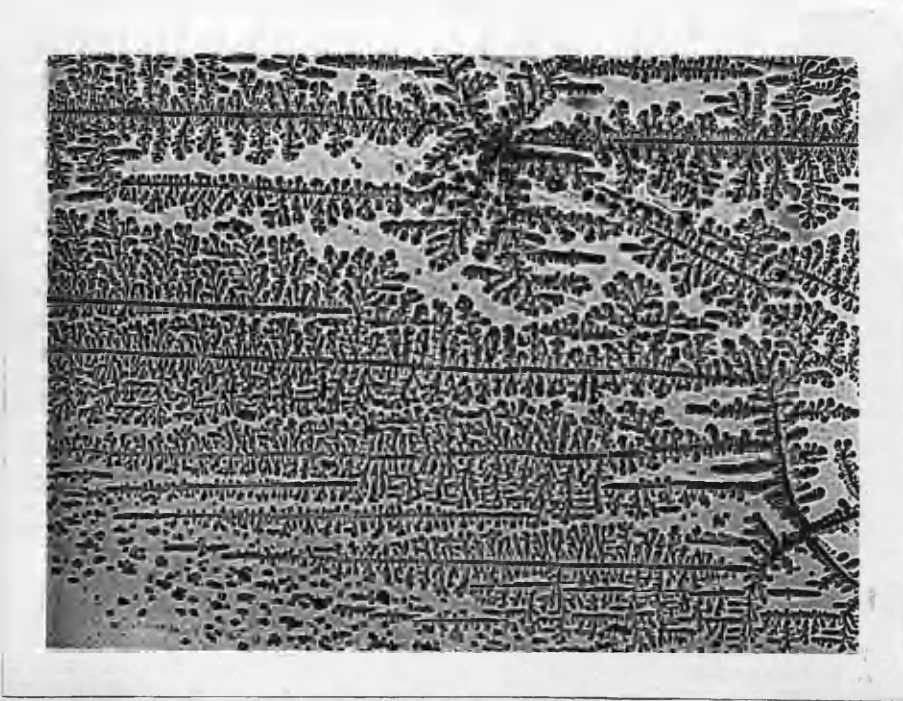


FIGURE 5.

X 100

Atypical crystals showing variations in the fern pattern.

first it was just a mass of jelly-like mucus, but as the water evaporated a few cells became obvious and then the crystals suddenly formed at one or two points growing rapidly, shooting out branches in all directions and producing a most exquisite pattern not only of ferns but also in floral, snowflake and leaf designs. A slightly different picture again could be produced by drying the slide rapidly over a bunsen flame: the crystals then arranged themselves in an interesting rosette or tree trunk formation. This method of drying the slides was quite convenient but it was found that the results were less accurate and so it was abandoned. Figure 5 shows one of the variations in the atypical patterns.

By studying the whole of the slide where typical ferns appeared it was seen that at the edges of the smear where the mucus had been spread more thinly the typical ferns gradually faded into the various atypical forms shown on Figure 4. Thus it was clear that the atypical forms did not represent any change in the composition of the crystals but merely variations in the concentration. Again it was clear that the smear had to be made quite thickly or a false diagnosis of reduced crystallization might be made.

Results - Correlation with Endometrial Histology and Abnormal Smears

In the 21 cases admitted to hospital for curettage, the mucous smear could be compared directly with the histological appearance of endometrium obtained on the same day or the following day. Eight of the smears were pre-menstrual, taken from patients complaining of infertility, and the remainder were scattered throughout the cycle,

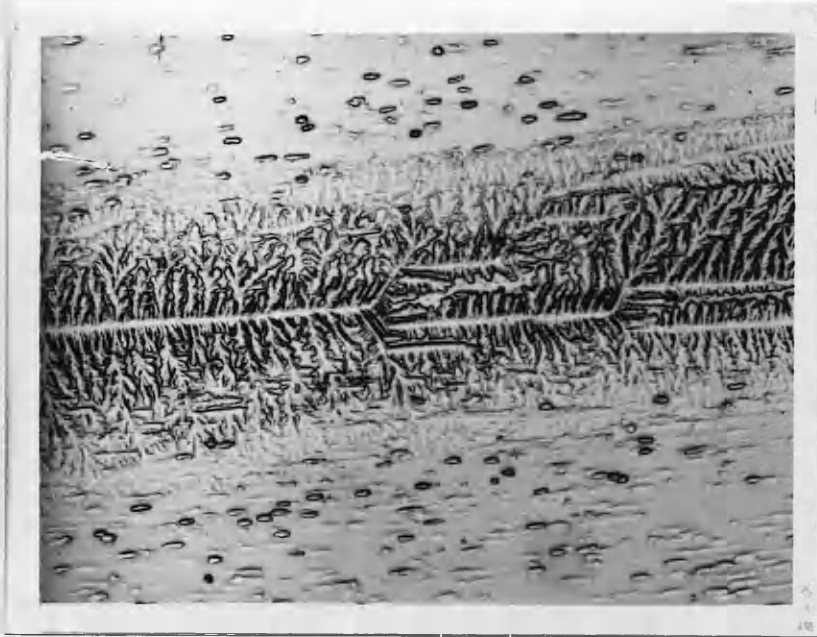


FIGURE 6.

X 100

Atypical crystals and endocervical cells.

Abnormal premenstrual mucus.

mainly from patients with mild dysfunctional bleeding.

Sixteen smears, including all those taken in the first half of the cycle, were normal and corresponded directly with the endometrial histology and the stage of the cycle. In the early proliferative phase the crystals were scanty or moderate, gradually increasing up to the strong typical crystals of Figure 2 immediately before secretory changes appeared in the endometrium. In the second half of the cycle as the secretory changes advanced, the crystals abruptly reduced, and disappeared about the 22nd day, leaving the premenstrual picture (Figure 3) of endocervical cells and no crystals.

The other 5 smears presented points of special interest. Two premenstrual smears unexpectedly showed moderate crystallization although the endometrium looked like normal secretory tissue: the assumption was that these patients had some minor form of hormone imbalance which showed in the mucus but not in the endometrium. Figure 6 illustrates one of the cases. Another 2 pre-menstrual smears also showed moderate crystallization but were associated with normal proliferative endometrium: here the assumption was that the patients were having anovulatory cycles. Finally one smear, about the 24th day, showed no crystals as expected but the endometrium was quite inactive.

Results - Post Menopausal Smears

Mucous samples were taken from 10 post-menopausal women. In 9 patients the mucus was found to be scanty, cloudy and very tenacious. The dried smear (Figure 7) showed simply a streak of mucus with no



FIGURE 7.

Inactive mucus with no crystals and no cells.
Normal post-menopausal smear.

recognisable cells or crystals. This confirms Papanicolaou's original observation and shows that the cervical mucus gives a good indication of ovarian function as well as oestrogen progesterone balance. One smear was taken from a post-menopausal patient who was subsequently found to have an endometrial carcinoma. The mucus was clearer and more elastic and the dried smear showed scraps of atypical ferns. This indicated residual oestrogenic activity since she had not been given oestrogens.

Results - Smears during Bleeding and with Inactive Cervical Glands

Further experience in taking smears during menstrual life showed that if the patient was actually bleeding at the time, then the crystals could be inhibited mechanically by the red cells. This is illustrated in Figure 8 where streaks of red cells break up the fern pattern. Thus, if the red cells were dense enough to make the slide look red to the naked eye, the smear was discarded.

Another problem was that the cervical glands in one patient seemed inactive and unable to respond to normal hormone stimulation. This fortunately is very rare but in such a patient there is no mucus to use for diagnostic tests.

Results - The Nature of Ferning

Early in the present investigation a colleague of the writer's examined a sample of typical ferning crystals by X-ray diffraction and was able to state that the material did in fact, consist of true crystals since it gave a sharply defined diffraction pattern. The method involves firing X-ray beams through the sample, photographing



FIGURE 8.

Moderate ferning, endocervical cells and red blood cells.
Mechanical disturbance of the ferning by the red cells.

the reflections off the atomic layers and then calculating mathematically the constituents and their relative proportions. He found that the crystals were composed almost entirely of a mixture of sodium and potassium chloride with the former predominant. The dendritic forms of silt crystals are similar but not identical, and it was clear that cervical mucous crystals are a mixture of sodium and potassium chloride with a very small amount of organic matter.

Literature and Discussion

A further search of the literature showed that Roland (1952) studied 20 normal parous patients and every second day throughout the cycle correlated a cervical mucus smear, endometrial biopsy and vaginal cytology. He found various forms of crystallization from the 5th or the 7th day through to the 20th or 22nd, but no crystallization premenstrually. In 30 patients known to be having repeated anovulatory cycles, crystallization persisted until the onset of menstrual bleeding. He suggested that crystallization of the mucus could be used as a simple test for the determination of ovulation and oestrogen activity. Zondek and Rozin (1954) in an excellent paper, indicated that ovulation could be shown by the mucus to have occurred in 72% of the cases making endometrial biopsy unnecessary in those cases. Pre-menstrual crystallization could occur in the presence of normal secretory endometrium and an anovulatory cycle therefore, could not be presumed. They stressed, however, in testing for ovulation that it was necessary to take two mucous samples, one in the late proliferative phase and another in the luteal phase, showing conversion from a positive to a

negative smear. A negative smear without the preceding positive would merely indicate a hypo-oestrogenic cycle, as in one of the writer's cases, or simply that the cervical glands were unable to respond to oestrogenic stimulation.

The suggestion by Roland that cervical mucus could be used to determine ovulation and oestrogen activity was quite sensible, but his observations were not entirely accurate. He thought, for example, that the quantity and type of fern would indicate the oestrogen level. This is true up to a point but since the type of fern is also influenced by the thickness of the smear, only a broad indication of oestrogen activity can be given. He also stated that pre-menstrual crystallization confirmed an anovulatory cycle; this is not necessarily true. The writer's findings are more in line with those of Zondek and Rozin.

There was considerable doubt expressed in the literature whether the ferning pattern seen in the dried cervical mucus represented true crystallization. Papanicolaou described it as "a kind of crystallization with aborisation. Rydberg (1948) compared it to fern or palm leaves and Zondek spoke of the "Palm Leaf" reaction. X-ray diffraction seemed the obvious way of determining the exact constituency of the material and by this method Zondek (1957) confirmed Rydberg's observation of a 97% sodium chloride content. The present investigation showed that the mixture was truly crystalline and composed not just of sodium chloride, but of sodium and potassium chloride with a very small amount of organic matter.

Zondek (1954) demonstrated that any non crystallising mucus could

be made to produce crystals simply by adding saline, showing that the presence of electrolyte was the decisive factor. In the second half of the cycle, progesterone presumably prevents the cervical glands excreting electrolyte and so the mucus does not normally contain crystals.

Conclusions

From this investigation it is clear that the cervical mucus provides a simple means of estimating hormone activity. Several definite conclusions can be made, each with a direct application in clinical practice.

1. Quite an accurate estimate of ovarian function can be made from the quantity and appearance of the cervical mucus alone.
2. The cyclical changes in the mucus and the presence of salt crystals and cells in the dried smear can be used to determine oestrogen activity and progesterone balance.
3. It can be presumed that ovulation has occurred if a mid-cycle crystallising smear is followed by a cellular non-crystallising pre-menstrual smear. If strong crystallization persists into the pre-menstrual phase an anovulatory cycle is very likely.
4. Salt crystals in a post-menopausal smear indicate persisting oestrogen activity and this may have some association with the development of carcinoma.
5. As a means of estimating hormone balance, examination of the cervical mucus is much less involved than urinary excretion methods and interpretation is much simpler than with vaginal cytology.

Summary

This preliminary trial emphasised the value of studying cervical mucous smears. A technique was established for obtaining the mucous samples, making dried smears and interpreting the results. The method proved to be very simple, and caused minimal disturbance for the patient.

The cervical mucus was found to vary throughout the normal menstrual cycle in quantity, appearance and viscosity. In the dried smear endocervical cells were seen before and after menstruation and a fern-like crystalline pattern appeared during the first half of the cycle, was maximal about ovulation time and faded again towards the end of the third week. This ferning was shown by X-ray diffraction to be composed of true crystals consisting of sodium and potassium chloride with a small amount of organic matter. Smears from normal post-menopausal women showed neither endocervical cells nor salt crystals.

The conclusion was reached that the mucus provided a simple clinical method of estimating ovarian function and oestrogen progesterone balance.

C H A P T E R III

PREGNANCY DIAGNOSISIntroduction

When he first studied cervical mucus, Papanicolaou (1946) noted that crystallization was absent in normal pregnancy. This at once suggested that the cervical mucus might be used in a simple clinical test for the diagnosis of early pregnancy, and several people carried out large series claiming to diagnose pregnancy from single mucous smears.

As one would expect, however, the normal pregnancy smear is identical with the normal pre-menstrual one (Figure 3), while patients near the menopause or with a tendency to hormone imbalance show variable and uncertain patterns. It seems unrealistic, therefore, to suppose that the appearance of a single smear should be diagnostic of pregnancy. Zondek (1954) found that he was unable to make a diagnosis by this method. He did show, however, that crystallising mucus could be produced by the administration of oestrogen in non-pregnant women, but not in pregnant women.

The writer decided that it would be useful to attempt the diagnosis of early pregnancy using cervical mucous smears after an injection of oestrogen.

Method

Two specimens of mucus were obtained from each patient by the method previously described. The dried smears were examined under the low power microscope and grouped as negative for crystals or

showing scanty, moderate or complete crystallization. Mucous samples were taken at the patient's first visit, 10 mg. of oestradiol monobenzoate or valerianate was given intramuscularly and the smears repeated at intervals up to 7 days. It was found that 10 mg. of oestradiol monobenzoate with a repeat smear on the 4th day gave the best results, and this regime was adopted as standard.

Investigation

To make the test as exacting as possible, only difficult diagnostic cases referred to the Gynaecological Clinic were chosen for study. Mucous smears were taken from 100 consecutive patients in whom the presence of an early pregnancy was uncertain. Some had previously been infertile with irregular menstruation, others were approaching the menopause, and another group had threatened to abort and it was not clear whether the pregnancy was still continuing. There were 43, 23 and 28 cases in those three groups and the remainder were stout patients in whom examination was unsatisfactory. These difficult cases requiring laboratory diagnostic assistance anyway were considered more suitable for trial than patients attending a normal ante-natal clinic. All the patients had less than 5 months amenorrhoea and the great majority less than 3 months: many were just a few days past their expected period. Aschheim-Zondek tests were also taken in 45 of the cases in order to make some comparison between the two methods of diagnosis.

All patients were followed until the diagnosis was clinically

TABLE 1

PREGNANCY DIAGNOSIS

<u>Test Result</u>	<u>True Diagnosis</u>	<u>Mucous Smear</u>	<u>No.</u>
Correct	Pregnant	Cells: no crystals	35
Correct	Non-pregnant	Typical or moderate crystals	41
False Positive	Non-pregnant	Cells: no crystals	2
False Negative	Pregnant	-	-
Doubtful	Pregnant	Cells: atypical crystals	21
Doubtful	Non-pregnant	Very few cells: no crystals	1
<u>TOTAL</u>			100

} 76%

} 22%

beyond doubt. Those who were pregnant were followed directly or through their doctors to delivery.

Results

Ultimately 56 patients were found to be pregnant and 44 not pregnant. The results are shown in Table 1. A correct diagnosis was made 76 times; there were 2 false positives, 21 doubtful pregnancies and one doubtful non-pregnancy. Out of the 45 Aschheim-Zondek tests performed at random on the same patients 6, or 13% were incorrect.

The 2 false positives were thought to have been due to persistent corpora lutea and when menstruation was delayed again in the same patients in a subsequent cycle, ferning appeared on the 4th day after oestrogen giving a correct diagnosis the second time. The tests from 21 patients who were subsequently shown to be pregnant were classed as doubtful. The slides showed a normal cellular pregnancy picture, but variable patterns of minor degrees of ferning also appeared in patches or streaks in a manner which could not be distinguished from a similar picture found pre-menstrually in some patients with hormone imbalance. One doubtful result in a non-pregnant patient was due to dysmucorrhoea, a fairly rare condition described by Zondek (1954) where the cervical glands seem unable to respond to hormone stimulation.

Literature and Discussion

Campos da Paz and Da Costa Lima (1953) took mucus from 182 pregnant patients and reported that there was no typical ferning in the smears but atypical ferning only ranging from 5% to 39% of cases during the first six months. They suggested that the only

fallacy in diagnosing early pregnancy from this test would be a persistent corpus luteum. Roland (1952) claimed a 99% success rate in diagnosis using mucous smears. He studied 300 pregnant patients and said that there was no ferning in the first three months with only negligible atypical ferning after 4 months. Accuracy of 99% was also claimed by Sprague (1954) who found only 5% atypical ferning in the first month.

For the reasons already given in the introduction to this chapter, single mucous smears are not diagnostic of pregnancy. This present series shows however, that the opposite can be true, namely that single smears can be diagnostic of non-pregnancy.

Zondek (1954) was the first to suggest the importance and value of giving oestrogen. He demonstrated that the cervical glands in pregnancy were hypertrophied and excreted more material, but despite large doses of oestrogen even up to 300 mg. of stilboestrol by mouth, they seemed unable to produce the type of mucus which contained electrolytes and, therefore, the mucus would not crystallize.

Conclusions

It was obvious that cervical mucous smears were less accurate as a pregnancy diagnosis test than the Aschheim-Zondek (76% compared with 87%). On the other hand, there are several advantages to be gained by examining the cervical mucus.

1. The test is simple to perform and interpretation is normally easy. If no crystals appear after oestrogen the patient is almost certainly pregnant, (only 2 incorrect in this series).

If profuse typical crystals appear on either slide as in metropathia haemorrhagica or with fibroids, a categorical diagnosis of non-pregnancy can be made at once.

2. A diagnosis can be reached in the clinic without reference to the laboratory, either immediately or in 4 days, while a gonadotrophin result normally takes 5 to 7 days.
3. The cervical mucus gives a diagnosis of pregnancy immediately after a missed period unlike the gonadotrophin test which does not become positive until 2 weeks later.

The final conclusion reached was that the gonadotrophin test was better in general for pregnancy diagnosis, but the cervical mucus provided a useful additional or alternative test.

Summary

A Pregnancy Diagnosis Test is described, using cervical mucus smears after an injection of oestrogen. The test was found to be less accurate than the Aschheim-Zondek gonadotrophin test, but it had certain advantages and was felt to be a useful additional or alternative test.



FIGURE 9.

X 100

Atypical crystals and endocervical cells.
Abnormal pregnancy mucus associated with abortion.

C H A P T E R IV

ABNORMAL MUCUS AND ABORTIONIntroduction

The crucial discovery in this work was made as is so often the case by simple observation. All the patients who were pregnant were followed either at the clinic or through their own doctors. It was quickly found that when the mucous test had been classed as doubtful in the early weeks because of the presence of atypical crystals, many of the patients aborted, sometimes months later.

Investigation

This observation was so striking and so important that a detailed study was made of these cases. Each patient's previous history was reconsidered and where the abortion had occurred in hospital, any clinical detail which suggested some reason for the abortion was noted. In addition, all the case notes were reviewed and the slides re-examined to see if there was any difference between the patients with ferning who aborted and those also with ferning who went to term without any disturbance.

In order to exclude the Pregnancy Diagnosis Test itself as a precipitating factor in the abortions, the other pregnant patients with normal non-crystallising mucus were also followed carefully. In theory it was possible that the injection of oestrogen might have contributed to the abortion in susceptible patients.

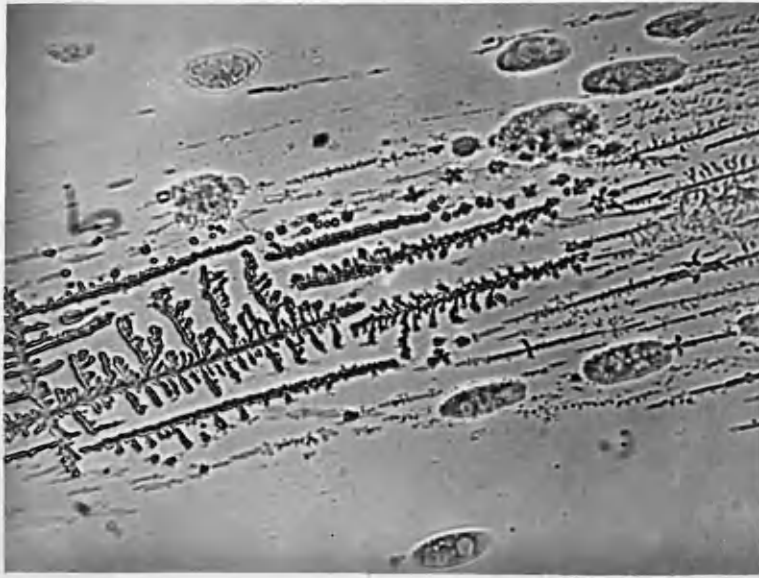


FIGURE 10.

X 400

Atypical crystals with a miniature fern pattern
(High power view of Figure 9)

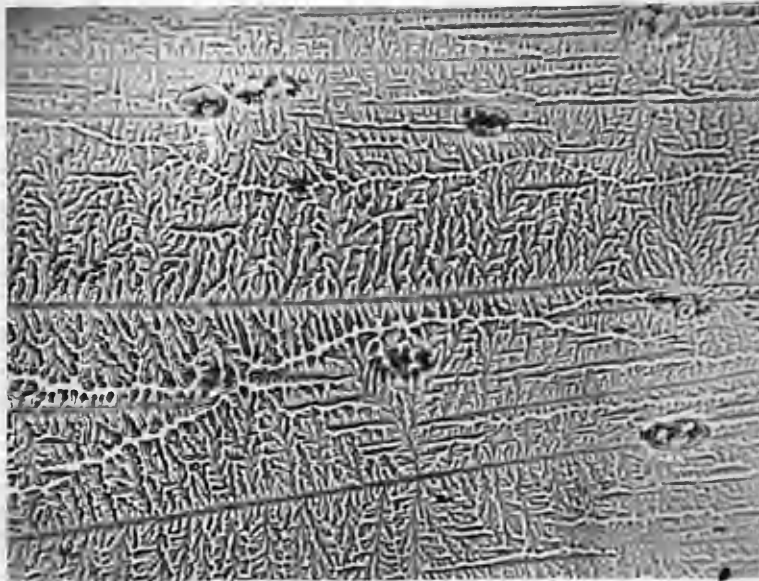


FIGURE 11.

X 400

Atypical crystals with a leaf pattern.
(High power view of Figure 9)

As more experience was gained over the years, it was appreciated that there was another type of abnormal smear in early pregnancy which was also associated with a high incidence of abortion. These cases received special study as well, and the results are reported under the heading of Ring Crystals.

Results - Abnormal Smears in Early Pregnancy

Out of the first 16 pregnant patients with atypical crystals in the mucus 8, or 50%, aborted, while the remainder continued to term and delivered normal infants. This suggested that the presence of salt crystals in the mucus in early pregnancy was indicative of some form of hormone imbalance which would lead to abortion in roughly 50% of cases.

Figure 9 shows a typical smear from one of the patients who aborted. There is a fairly normal pregnancy pattern with rather fewer cells than usual, and in places as in the photograph a central streak of salt crystals in miniature fern pattern. Figures 10 and 11 show the same slide under higher magnification and it is clear that the crystal formation is basically the same in smaller form.

On the other hand, there were 8 patients with similar smears who did not abort. Their slides were re-examined and compared with those of the abortion patients. Figure 12 shows a patient who had quite marked ferning as late as 15 weeks and had an uneventful pregnancy. No difference could be found in the quantity, appearance or viscosity of the mucus, in the degree of cellularity, or in the amount and type of ferning.

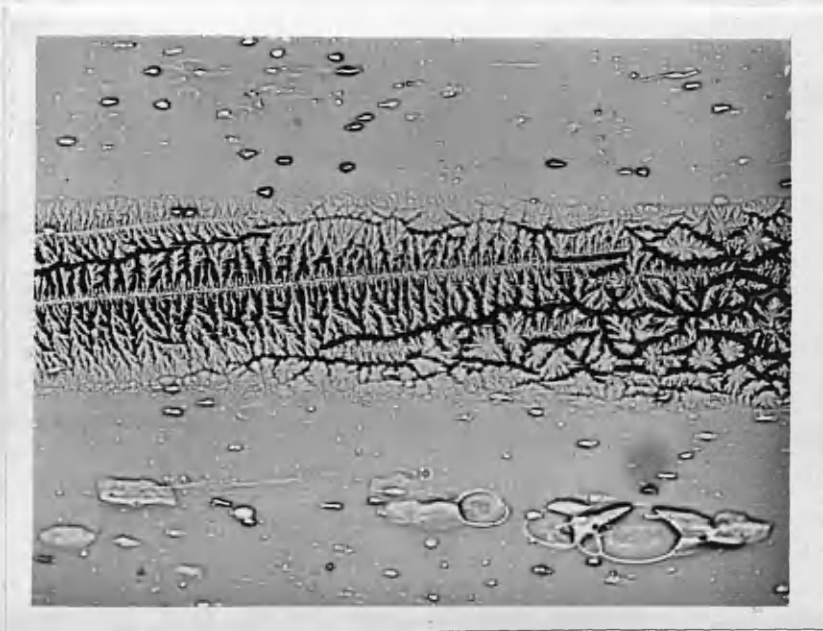


FIGURE 12.

X 150

Moderate ferning and scanty endocervical cells.
Abnormal mucus at 15 weeks but uneventful pregnancy.

Significantly however, the histories showed that only 2 patients with previous successful pregnancies came into the abortion group; the other 6 had been infertile or had had previous abortions. Six of the patients with ferning who did not abort had previous good histories.

Results - Normal Smears in Early Pregnancy

In the Pregnancy Diagnosis series of 100 cases there were 35 pregnant patients with normal mucous smears showing endocervical cells and no crystals (Table 1). All these patients were followed to delivery and their abortion rate was 6.9% which is rather less than the general average and shows the marked difference between the two groups with normal and abnormal mucous smears.

Results - Ring Crystals

Many more patients were seen as the investigation continued but it was not until 1959 that it was appreciated that salt crystals were appearing occasionally in another form quite different from the usual ferning. This observation arose out of a special study of the slides from a few patients whose smears showed insignificant ferning or no crystals at all, but who subsequently aborted.

One slide which had contained minimal ferning was re-examined some time later, and it was found that the ferning had disappeared and had been replaced by numerous dark deposits of crystalline material giving an appearance of rings under the microscope. Figure 13 shows these rings under the low power and Figure 14 is the same slide under the high power. On another slide, the density of ring crystals was

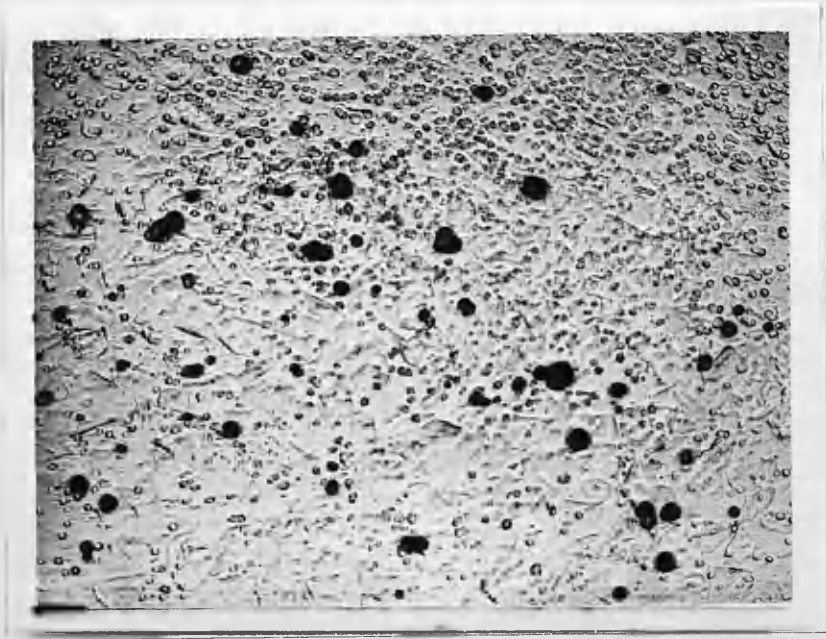


FIGURE 13

X 100

Ring crystals, squamous cells and endocervical cells.
Abnormal pregnancy mucus associated with abortion.

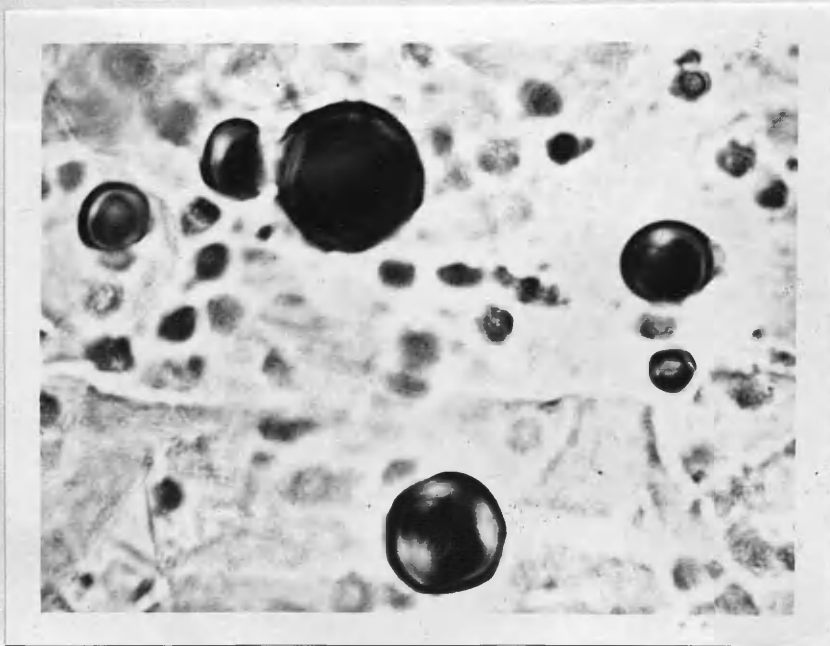


FIGURE 14

X 400

Ring crystals. (High power view of Figure 13).

such that they grouped themselves together in a fern-like mass (Figure 15) and it is clear that these rings are basically similar to the ferning crystals. Presumably the proportions of the constituents are slightly different and so the fern pattern is not formed.

Altogether 15 pregnant patients have been found to date with significant ring formation but no ferns. Seven of these aborted including 3 who had a clinical diagnosis of missed abortion and one where there was no foetus in the sac. The prognosis therefore, in the presence of ring crystals is as bad as with ferning, while any form of treatment has less hope of success because of the possibility that the pregnancy has already stopped growing.

Subsequently rather faint ring crystals were found quite commonly round the periphery of normal ferning, and in those cases the prognosis was as expected for the ferning. Figure 16 shows normal cells with a streak of faint ring crystals in the upper portion of the picture. The appearance of these faint rings suggest that they are normal cells lightly covered with some crystalline material. It is in cases which show extensive ring formation without ferning that the prognosis is so uncertain.

Literature and Discussion

Subsequent review of the literature showed that as early as 1946, Papanicolaou, discussing the value of vaginal smears in pregnancy, said that one could recognise some abnormal features suggestive of threatened abortion such as excessive mucus. Clift, Glover and Scott Blair (1950) studying cervical mucus rheology mentioned that some pregnant women

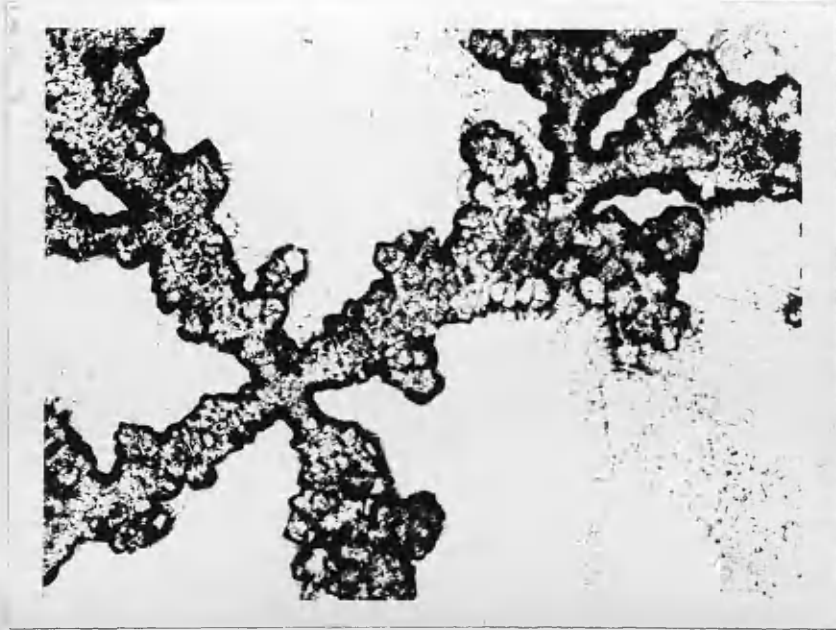


FIGURE 15.

X 125

Ring crystals grouped into a fern-like mass.

whose cervical secretions were less viscous than expected were later known to abort. Zondek and Cooper (1954) examined mucus from 226 women in the first trimester and found 26, or 11%, with scanty or moderate ferning. Independently these writers made the same observation as in the present investigation, namely that ferning in early pregnancy was associated with roughly a 50% risk of abortion. Zondek, Foreman and Cooper (1955) reported that 15 of these 26 cases terminated unfavourably: 2 patients had tubal pregnancies and 13 aborted. They suggested that ferning in pregnancy indicated a degree of placental insufficiency which might be reversible or irreversible.

Three problems had to be considered:-

1. What form of hormone imbalance did the crystals in pregnancy represent?
2. What therapy could be given to correct this imbalance?
3. How could the 50% of patients destined to abort be selected from the other 50% who did not require treatment?

Crystallization in pre-menstrual mucus had been shown from endometrial histology to be associated with defective luteinisation or insufficient progesterone. By analogy, therefore, it was reasonable to suppose that crystallization in pregnancy mucus might be associated with a relative progesterone deficiency. Zondek and Cooper (1954) thought that the absence of crystals in normal pregnancy mucus could not be due to progesterone and, therefore, the presence of crystals could not be due to progesterone deficiency. This was based on the argument that pregnanediol excretion was not greatly increased in

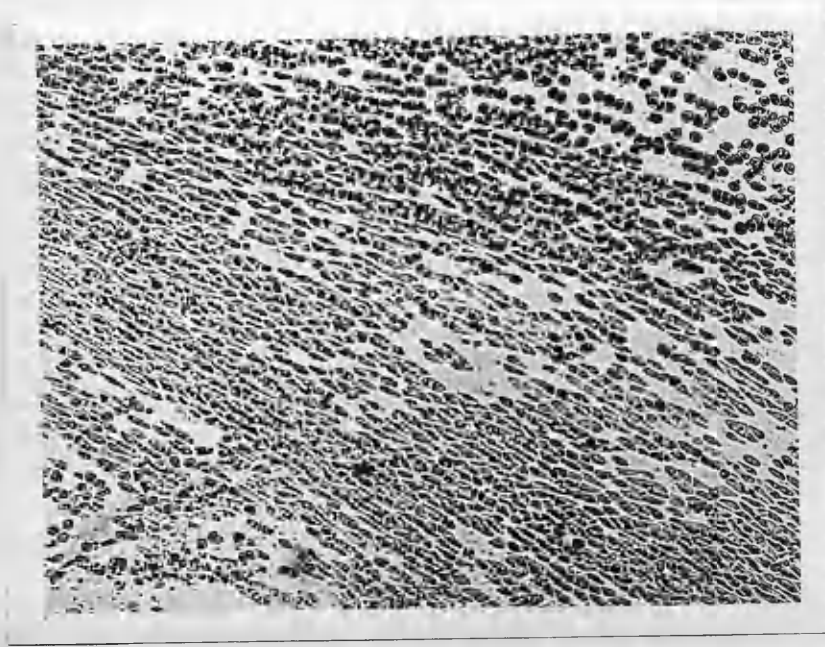


FIGURE 16.

X 100

Endocervical cells with rather faint ring crystals.

early pregnancy and yet, very large doses of oestrogen, up to 13 g. of stilboestrol by mouth, did not produce crystals in normal patients. Moreover, in some cases of missed abortion with low pregnanediol levels, they found that the mucus remained in a non-crystallising state for weeks or even months as long as the dead foetus remained in utero. They concluded that during normal pregnancy it was not increased progesterone production which prevented crystals appearing but a specific inability of the cervical glands to allow the permeation of electrolytes. Pregnanediol excretion, however, is not necessarily a true indication of progesterone activity, since many substances can be given which exert a progestogenic effect without raising the pregnanediol level. Again stilboestrol given by mouth in pregnancy may be inactivated quite quickly in the liver and so exert a limited effect. The writer's observation of the ring form of crystals may also help to explain Zondek's problem about the absence of ferning crystals in some cases of missed abortion. Mucus from patients with missed abortions regularly shows salt crystals but in varied forms.

The reason for the appearance of crystals in some pregnancy mucus remains uncertain, and the final explanation will probably need to wait for more extensive blood and urinary hormone studies. It is, however, quite easy to show in non-pregnant patients that the administration of progesterone will convert proliferative endometrium to secretory tissue and abolish salt crystals in the mucus. It seemed logical to the writer to consider giving progesterone to pregnant patients with abnormal crystallising mucus. If the mucus could be

converted to normal viscosity and appearance then presumably this would indicate that the hormone imbalance had been corrected and the risk of abortion reduced. Pierce and Cope (1955) working on the same theory rather in reverse, argued that ferning suggested a progesterone deficiency and that such women might, therefore, have a greater tendency to abort. They examined 100 pregnant women: 17 had ferning and 3 of those aborted (17%), whereas 6 of those without ferning aborted (7%).

Ullery and Shabbanah (1957) thought that the degree of cellularity would indicate the patients with irreversible changes but no one else since then has been able to confirm this. In the present series the degree of cellularity varied considerably but there was no constant trend on which to base any prognosis. It was clear, however, from studying the case records, that those with a previous poor history, such as prolonged infertility or recurrent abortions, were more likely to abort than those with two or three previous successful pregnancies. This was logical enough and suggested that the combination of a previous poor history with recurrent ferning in early pregnancy should be used as means of selecting patients who needed treatment.

These observations were made in 1956 and in the following year Jacobson (1957) reported similar conclusions from America. He thought that cervical mucous smears in pregnancy provided a good method of identifying women likely to abort. Successive non-ferning smears indicated a normal pregnancy, but positive smears with an adverse medical history or in threatened abortion were a definite indication for progesterone therapy.

It is difficult to know what proportion of all abortions is formed by those with hormone imbalance and ferning in the mucus. The patients seen in this series are not a normal cross section of the pregnant population, but some idea of the relative proportions is suggested in a paper by Efstation and Schwalenberg (1960). They took smears from 500 pregnant patients and found that 11% of the smears showed some crystallization: there were 35 abortions, an incidence of 7%. Ferning smears had been obtained from 15 of the patients who aborted, and negative smears from the other 20. In each case where crystallising smears were associated with abortion there was said to be histological evidence of decidual or chorionic abnormality. The causes of the abortions among the patients with negative smears were said to be incompetent os, agenesis of the foetus, cord complications, uterine anomalies, criminal abortions and 8 unknown. From these figures it would appear that roughly 40% of abortions are associated with salt crystals in the mucus.

Conclusions

1. The presence of salt crystals in early pregnancy mucus is associated with a 50% risk of abortion.
2. Repeated negative smears with no salt crystals indicates a normal pregnancy with less than average risk of abortion.
3. Salt crystals in early pregnancy suggest a degree of hormone imbalance. By analogy with premenstrual conditions this may be a relative progesterone deficiency.

4. Patients with a previous poor history and recurrent crystallising smears require treatment, and should be selected for therapy on these criteria.

Summary

It was discovered that the presence of ferning or ring crystals in early pregnancy mucus was associated with a high incidence of abortion, while patients with normal non-crystallising smears were less likely to abort than the average. The significance of the observation was discussed and the conclusion was reached that patients should be selected for treatment with progesterone on the combination of a previous poor history and recurrent crystallising smears.



FIGURE 17

X 150

Case 39. Moderate ferning.
Progesterone therapy and successful pregnancy.

CHAPTER V

PROGESTERONE THERAPYIntroduction

As soon as it was appreciated that pregnant patients with salt crystals in the mucus had a 50% risk of aborting, an urgent need was felt to attempt some form of treatment in subsequent patients in order to reduce this risk. It had been argued that the crystals indicated a relative progesterone deficiency, and so a clinical trial was undertaken giving intensive progesterone therapy to selected patients.

Method - Diagnosis

Smears were taken at weekly or fortnightly intervals by the method previously described and definite crystals had to be seen on 2 occasions before treatment was considered. The Hogben test was used to confirm the diagnosis of pregnancy in each case unless previous abortions had occurred before 7 weeks, and in such cases treatment was started as soon as crystals were seen after a missed period. In this way it was ensured before treatment began, that each patient was pregnant and had a persistent hormone imbalance.

Method - Type of Progesterone

Right at the beginning of the investigation it was decided that some form of progesterone which could be given by injection would prove to be the most satisfactory. Oral therapy has the disadvantages that the tablets may be forgotten or simply not taken and that the

material can be lost by vomiting, or failure of absorption. Injections may be uncomfortable but at least the treatment is known to have been given and none of our patients objected.

17-alpha-hydroxy-progesterone caproate (Primolut Depot) was chosen since it was the only injectable progestogen of high potency. It had the additional advantage that a single injection was known to provide a steady hormone level over a period of at least a week and so treatment could be given at weekly intervals.

The doses used initially ranged from 30 mg. to 125 mg. but the work of Greenblatt (1956) and Davis and Weid (1957) suggested that this was inadequate. The quantity of hydroxy-progesterone caproate required to convert oestrogen-primed endometrium to full secretory tissue is 250 mg. and it was decided to use this as the basic weekly minimum dose. Many patients were given 500 mg. a week if the crystals were slow to clear from the mucus and the maximum amount used was 500 mg. twice a week over a period of two months given to one patient who had recurrent threatened abortions while on the low dosage.

None of the patients noticed any toxic effects at all. One patient did complain of local discomfort at the site of injection with a surrounding area of erythema, but both the discomfort and the erythema were subsequently prevented by giving her an anti-histamine tablet half an hour before the injection.

One of the problems of progesterone therapy in early pregnancy is the risk of producing virilization in a female foetus. There were,

however, 8 female children born to patients treated in this series and all were normal.

Investigation - Preliminary Trial

As soon as the decision was made to start treatment, the next 6 patients in the first trimester of pregnancy showing ferning in various degrees were given progesterone in a variety of doses as described below. The first patient started with oral therapy but thereafter the progesterone was invariably given intramuscularly. At the same time, serial urinary pregnanediol estimations were made on 5 of the treated patients and 2 other non-treated patients who also showed similar ferning.

Investigation - Subsequent Selection of Cases

This preliminary trial of the value of progesterone in selected cases was so successful that it was decided to undertake a long term study on the same basis. Patients were selected as requiring treatment on the criteria of a previous bad history and recurrent slides showing scanty or moderate but definite ferning.

Two or three previous consecutive abortions or one previous abortion with prolonged infertility were thought sufficient to justify treatment if the smears contained crystals. Simple threatened abortions in the first pregnancy and usually in the second were not treated even if crystals were present. If the previous abortions had occurred after 15 weeks maturity it was considered that hormone imbalance was not likely to be the cause and such patients were not

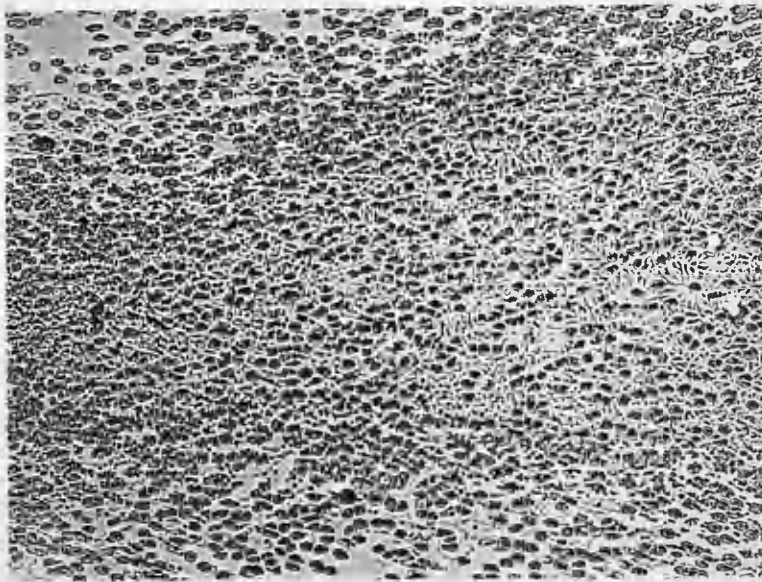


FIGURE 18

X 100

Case 47. Faint leaf pattern of crystals.
Progesterone therapy and successful pregnancy.

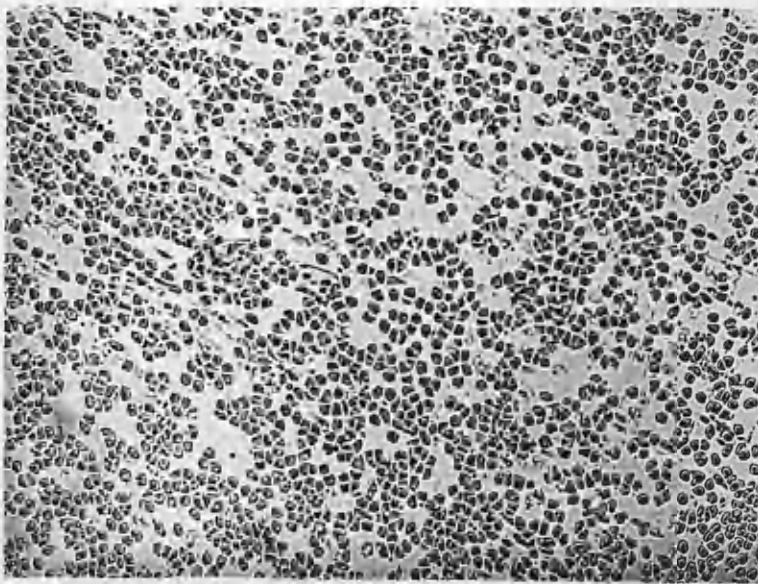


FIGURE 19

X 100

Case 47. Faint rings only at 12 weeks.

treated. Provocation with oestrogen to confirm the pregnancy was discontinued. There was no suggestion that any of the earlier cases given oestrogen had aborted as a result, but there was a theoretical risk involved of aggravating a pregnancy which might already be disturbed.

Altogether 191 patients suspected of being in early pregnancy were seen and examined at least twice. One hundred and eighteen were found to be pregnant and the remaining 73 who were not pregnant, were returned to their own doctors once the diagnosis had been confirmed. Sixty-five of the pregnant patients, or a little over 50%, had some degree of ferning in the cervical mucus, and details about these cases are given in the section on Results. Four of the 65 patients who showed ferning could not be traced right through to delivery and they have been excluded, leaving 61 patients for special study.

From this special group of 61 pregnant patients with crystals in the mucus, the first 16 have already been mentioned: 8 aborted at varying intervals after being seen and the other 8 had normal babies at term. The next 6 patients were treated as described below and thereafter cases were selected for treatment strictly on the criteria of recurrent crystals with a previous poor history. Since then a further 14 patients have been given progesterone making a total of 20 patients treated to date. The remaining 25 patients were followed to delivery but no special treatment was given.

Results - Preliminary Trial

Six patients were included in the preliminary trial and the

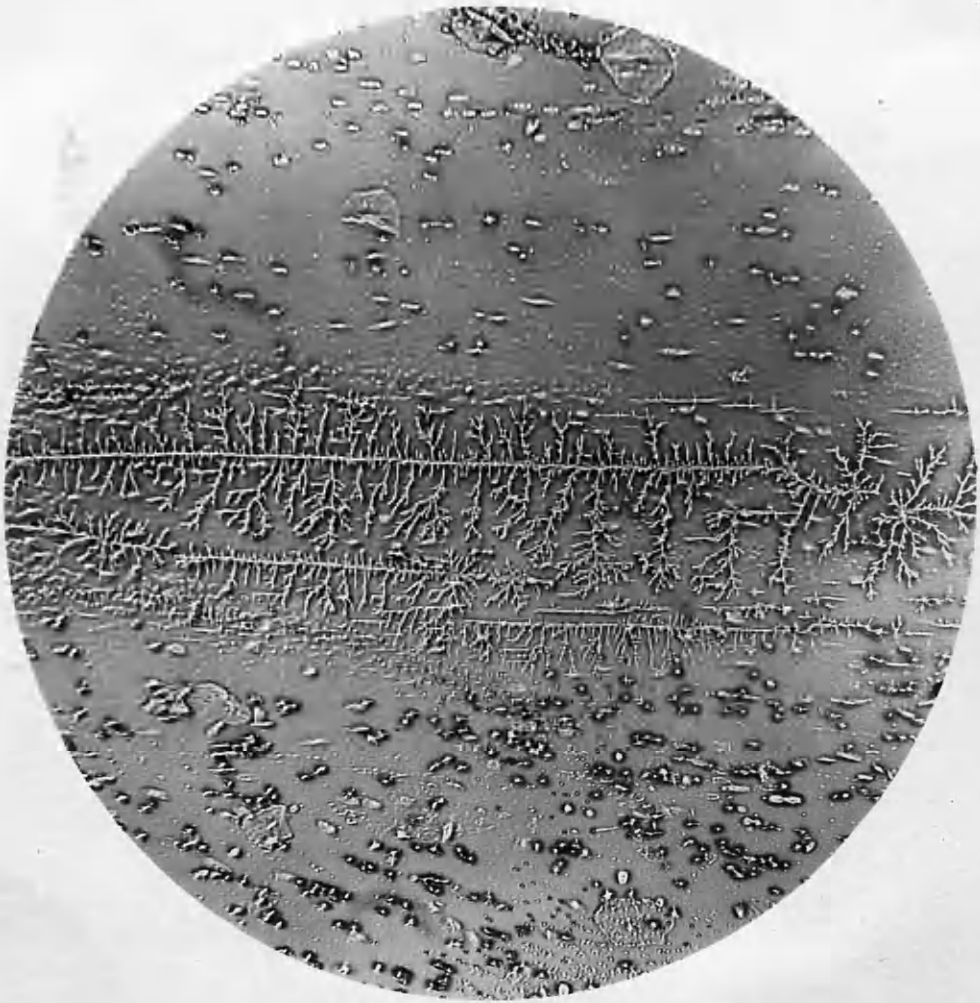


FIGURE 20.

X 140

Case 51. Habitual abortion. Moderate ferning.
Progesterone therapy and successful pregnancy.

first 3 cases are worth recording in some detail.

Case 1. Mrs. A.M., aged 28, had been previously investigated for infertility and was said to have uterine hypoplasia. There was moderate ferning at 9 and 11 weeks pregnancy, and she was given 30 mg. of ethisterone daily by mouth. Ferning became negative at 13 weeks and the progesterone was changed to 65 mg. long acting solution by injection monthly, and was then discontinued at 22 weeks. Pregnanediol estimations averaged the very low figure of 2 mg. per day at 11 weeks rising to 14 mg. per day at 22 weeks. The patient went into premature labour at 32 weeks and delivered a 21b.15oz. boy who survived.

Case 2. Mrs. V.McC., aged 30, aborted in her first pregnancy at 12 weeks. Myomectomy was performed for a single intramural fibroid. In the second pregnancy there was scanty ferning at 6, 9 and 11 weeks. No treatment was given and she aborted at 6 months. She became pregnant once more and had similar ferning at 8 weeks. Long acting progesterone 125 mg. was given by injection monthly to 26 weeks. Pregnanediol estimations averaged 10 mg. per day at 8 weeks in the second pregnancy which ended in abortion compared with 6.4 mg. per day at 8 weeks in the third pregnancy before treatment began. She had a threatened abortion at 24 weeks which settled with rest and then went through to term and delivered a live 8lb. male child who was known to be thriving a year later.

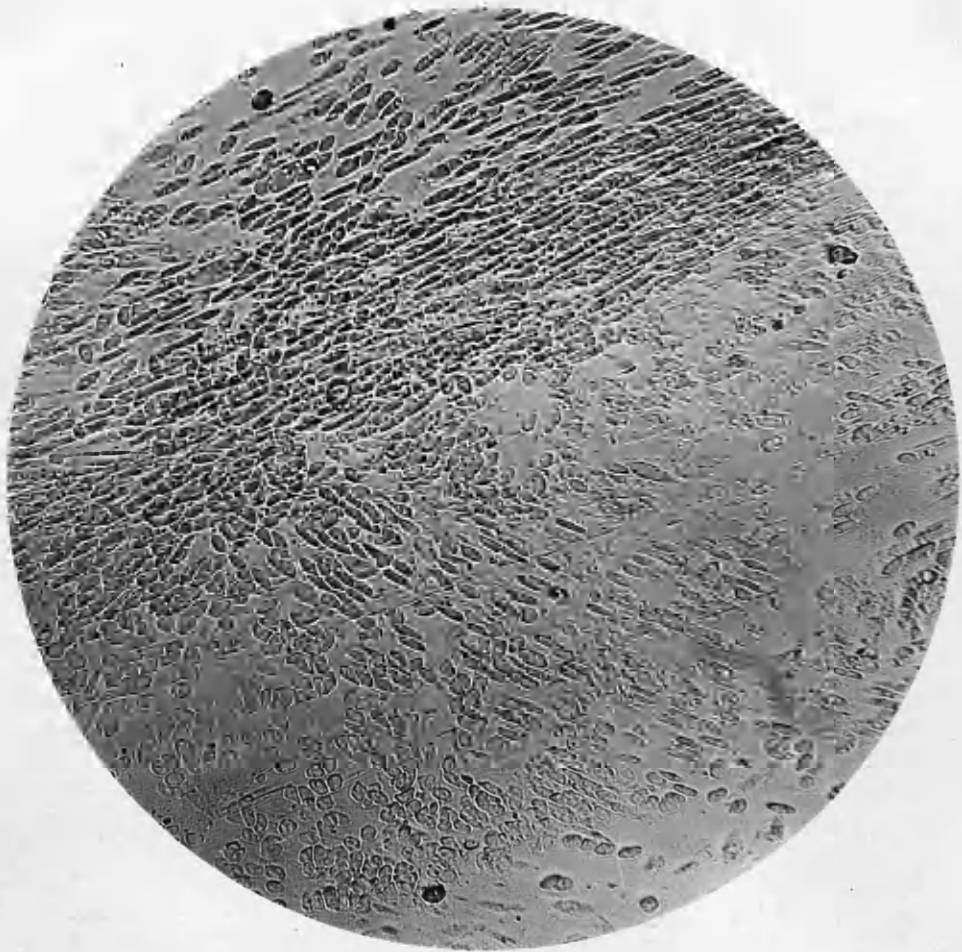


FIGURE 21.

X 140

Case 51. Endocervical cells only at 15 weeks.

Case 3. Mrs. B.M., aged 26, had a threatened abortion at 6 weeks. Moderate ferning was found at 10, 11 and 13 weeks. Thirty mg. of long acting progesterone was given weekly to viability. Pregnanediol estimations averaged 9.5 mg. per day at 11 weeks. The ferning became negative at 18 weeks and the patient had a normal delivery at term. Subsequently she moved to another part of the country and had an abortion at 12 weeks maturity 18 months later.

Of the 6 patients treated with progesterone 5 went to term, one laboured prematurely at 32 weeks and all have surviving children. The psychological effect of hormone injections is, of course, considerable, but the way in which the crystals diminished and disappeared and the successful outcome in each case did suggest that the progesterone might be of specific benefit.

Results - Urinary Pregnanediol Estimations

The pregnanediol estimations on the whole were disappointing. The readings generally were on the low side but there was no obvious correlation between the pregnanediol level, the amount and type of ferning or the outcome of the pregnancy. In one of the untreated cases an average reading at the reasonable level of 10 mg. per day at 8 weeks was followed by abortion, but in the other untreated case an average reading of 7.5 mg. per day at 8 weeks was followed by a normal pregnancy and delivery. Among the treated cases the readings ranged from 2 mg. per day to 9.5 mg. per day at

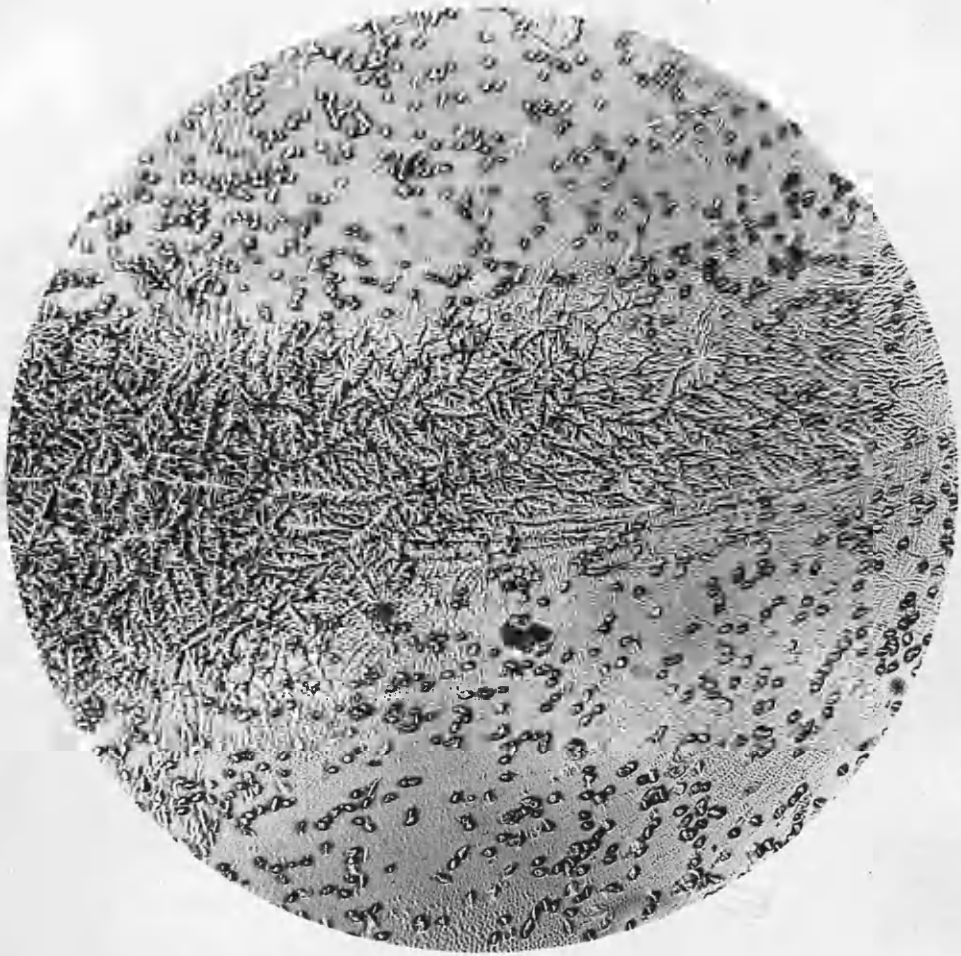


FIGURE 22.

X 140

Case 56. Previous abortion and infertility.
Moderate ferning at 7 weeks.
Progesterone therapy and successful pregnancy.

the start of treatment between 8 and 11 weeks. The general impression gained was that the pregnanediol estimations were not worth doing and we should rely on the simpler and quicker ferning tests.

Results - General Distribution of Cases

One hundred and eighteen of the patients examined were found to be pregnant, and 65 of those, or a little over 50%, had some degree of ferning in the cervical mucus. This is a higher percentage of patients with ferning than other investigators have recorded, but it can be explained quite reasonably by the type of patient seen at the clinic, many of them being referred specially because of previous abortions and infertility. There was no ferning in the mucus from 38 pregnant patients and they were classed as normal. Significant ring type crystals without ferns were found in 15 patients, and these cases were described in the previous chapter. Detailed results are given below.

Results - Normal Cases without Ferning

Pregnant patients without ferning in the mucus have been classed as normal. Three out of the 38 patients aborted and there was one ectopic pregnancy, an overall loss of 10.5% which is probably average or a little less than average for the country as a whole. Two of the abortions had maternal causes, influenza and severe heart disease, and the third was associated with complete absence of an embryo in the sac.

Results - Ferning Cases Treated Successfully

Case 39. Figure 17 shows one of the cases treated with 250 mg. of



FIGURE 23.

X 140

Case 56. Dense moderate ferning at 7 weeks.
(Same mucus as Figure 22)

progesterone caproate weekly. The ferning disappeared after two injections and she had a spontaneous delivery at term after 2 small ante-partum haemorrhages for which no cause was found. The female child weighed 7lb.10oz. and was quite normal.

Since one of the previous patients had laboured prematurely at 32 weeks when treatment had been stopped at 22 weeks, the injections were continued up to 34 weeks with all subsequent patients. In the future it may be reasonable to give oral progesterone to selected patients after 16 weeks, but it would seem sensible to continue some form of therapy until the risk of prematurity is passed.

Case 47. Figure 18. shows a faint background leaf pattern of crystals in a patient who had had 3 previous abortions at 10 weeks without explanation. The crystals reduced to faint rings only at 12 weeks (Figure 19) and then vanished, but recurred as tiny fern patches at 14 and 16 weeks. The progesterone was increased to 375 mg. then 500 mg. weekly and she had an uneventful pregnancy, delivering a 6lb.4oz. boy quite normally at 39 weeks. No further crystals were seen after the dose was raised to 500 mg.

Case 51. A more recent patient who had had 3 previous abortions at 3 months is illustrated in Figure 20. Progesterone caproate 250 mg. was given weekly and the ferning had cleared by 15 weeks (Figure 21). The patient went on to have a forceps delivery of a normal baby girl weighing 6lb.11oz. at 41 weeks.

Case 56. Another recent patient had had one previous abortion and a long period of infertility. Figures 22 and 23 show different

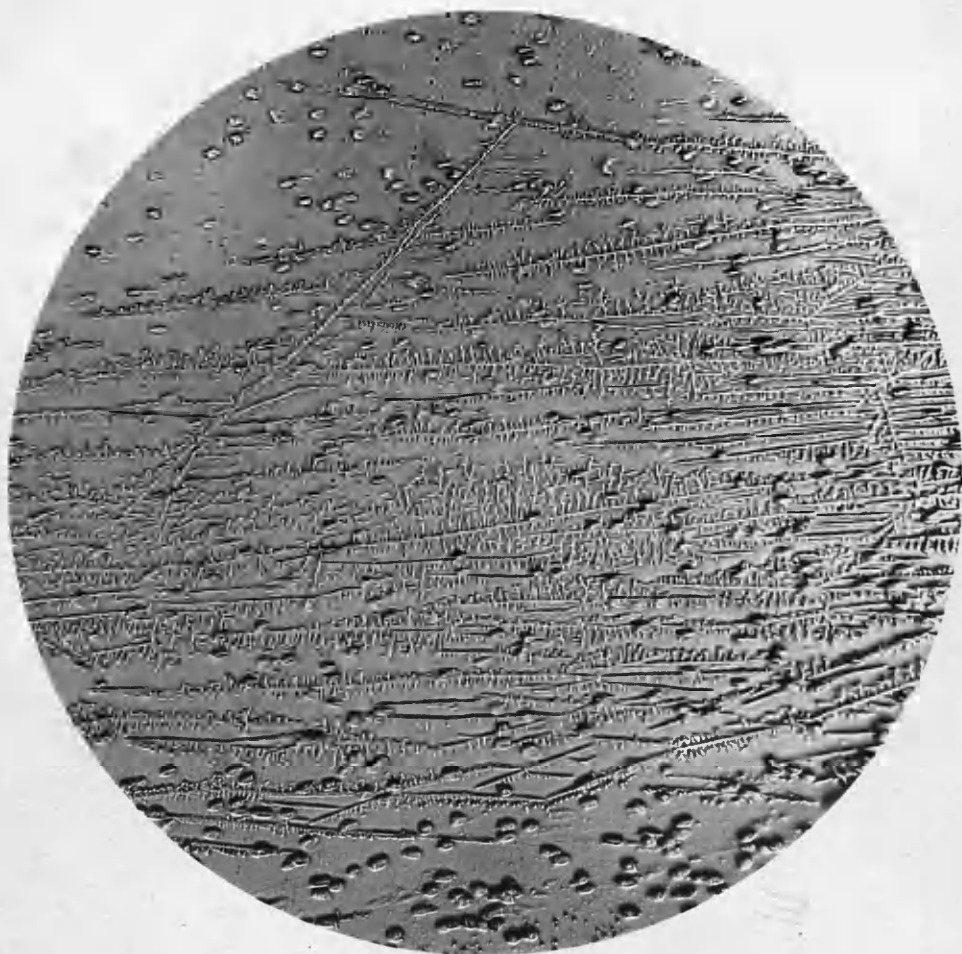


FIGURE 24.

X 140

Case 57. Previous normal pregnancy.
Moderate ferning.

No treatment and normal delivery

areas of slides taken at 7 weeks. Progesterone caproate 250 mg. was given weekly to 34 weeks. The crystals gradually reduced and disappeared at the 13th week. She delivered a normal female child weighing 6lb.4oz. at 39 weeks but the placenta weighed just 1lb.

Case 57. The difficulty of deciding whether treatment is needed from the slide alone is well illustrated by Figures 24 and 25 which are quite similar to the two previous photomicrographs. Because this patient had had a normal pregnancy before, no treatment was given and she had a normal delivery at term with a 16oz. third stage haemorrhage.

The principle is now well established that there must be a combination of recurrent positive smears with a previous poor history before treatment is considered necessary.

Results - Recurrent Abortions Despite Treatment

Two cases were classed as failures.

Case 45. This patient had had 3 previous abortions, once at 13 weeks and twice at 6 weeks. Ferning smears were found at 9 weeks (Figure 26) but there were red cells on the slides as well. Progesterone caproate 250 mg. was given weekly and the amount of ferning seemed to reduce (Figure 27), but it recurred at 12 weeks and she aborted as before at 13 weeks. Although the uterus had appeared to be getting bigger, the foetus was only 8 weeks in size and the final conclusion was



FIGURE 25.

X 100

Case 57. Dense moderate ferning.
(Same mucus as Figure 24).

that it had stopped growing before treatment began.

Case 62. The other unsuccessful case was a patient who had had 3 previous abortions at 10, 13 and 14 weeks without obvious reason. Ferning smears were found at 6 and 7 weeks in the 4th pregnancy and 250 mg. progesterone caproate was given weekly. The crystals cleared after 2 injections but she complained of a persistent brown discharge and aborted again at 11 weeks. Clinically it was a missed abortion of 6 to 8 weeks size and the membranous sac and chorionic tissue were sent for histology. The report was conclusive that no foetus had formed within the sac and the final diagnosis was of slight hormone imbalance together with foetal agenesis.

Although these two cases were frankly disappointing, particularly in view of their previous distressing histories, they did not reduce the value of the rest of the work. The method of selection and treatment proved successful where hormone imbalance was the only abnormality.

Results - Persistent Ferning

The crystals normally cleared from the mucus before the 15th week and very often one or two injections of progesterone. In one patient, however, there was no significant change in the mucous picture despite the largest doses of progesterone given in this series.

Case 61. This patient had had 2 previous abortions at 12 weeks with no obvious cause. There was quite widespread ferning at



FIGURE 26.

X 100

Case 45. Habitual abortion.
Moderate ferning and faint ring crystals at 9 weeks.

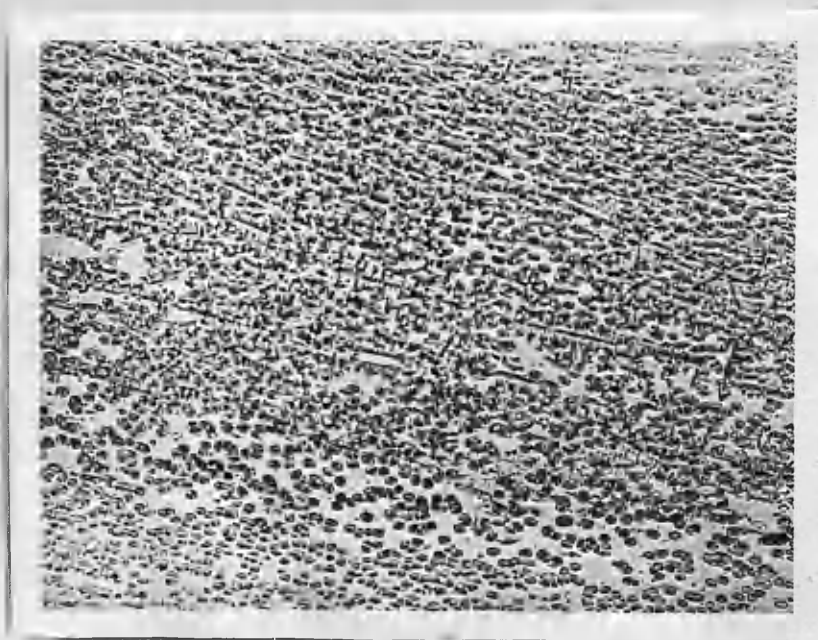


FIGURE 27.

X 100

Case 45. Scanty ferning and faint ring crystals at 11 weeks.
Abortion again despite progesterone therapy.

6 weeks (Figure 28) and 250 mg. hydroxy-progesterone caproate was given weekly. By 10 weeks there was no change and the amount was increased to 500 mg. She was admitted for rest at the 14th week after a slight fresh blood loss, and when it was found that the amount of crystallization had actually increased, the progesterone was raised to 500 mg. twice a week. There were further slight fresh losses at 16 and 19 weeks, but thereafter she remained well. By 26 weeks the crystals had diminished but were still present. The failure to convert positive to negative smears has not yet been explained, but the patient has now reached 31 weeks maturity and the placenta may give some more information when she delivers.

Case 60. An almost identical smear (Figure 29) was obtained from another patient being treated simultaneously with the same batch of progesterone. She pursued a normal course with negative smears from 13 weeks and had a normal delivery at 38 weeks. The progesterone itself, therefore, was not at fault.

Results - Third Stage Complications

Because of the association between the crystals in the mucus and a tendency to abortion, it was natural to look for third stage complications in those with ferning who went to term, on the assumption that there might be chorionic or decidual abnormalities.

TABLE 2

ABORTION RESULTS

<u>Diagnosis</u>	<u>Mucous Smear</u>	<u>Number</u>	<u>Management</u>	<u>Abortions</u> <u>No.</u> <u>Per Cent</u>
Not pregnant	Typical or moderate crystals	73	Returned to their own doctor	- -
Normal pregnancy	Cell: no crystals	38	No treatment: followed to delivery	4 10.5%
Abnormal pregnancy	Ring crystals	15	No treatment: followed to delivery	7 46.7%
Abnormal pregnancy	Cells: atypical or "Ferning" crystals	65	Initial 16 cases - no treatment 4 no treatment - lost track 20: Progesterone therapy	8 50.0%
			25: Previous good history: no treatment	- -
				2 10.0% } 13.3%
				4 16.0%
Total Cases seen		191		

However, only 5 of the 61 patients with ferning who were traced through to delivery had a blood loss of over 10oz. or a retained placenta. There were 3 treated patients and 2 untreated ones, an overall incidence of 8.2% which is not significant. Histological reports are being obtained on as many of these placentae as possible but so far no distinctive abnormality has been found.

Results - Untreated Ferning Cases

There were 4 abortions from the 25 patients with ferning who were not treated, an incidence of 16%. Treatment was not given to these patients because they were in their 1st or 2nd pregnancy or had previously had a normal delivery. Of the 4 abortions one proved to be inevitable and one missed. The 3rd patient had had an abortion before when no cause was found and it was decided she should have progesterone if ferning recurred another time. The 4th abortion was associated with a very much unwanted pregnancy. The abortion rate for all the patients with crystals in the mucus was, therefore, reduced from 50% before selection and treatment to 13.3% (Table 2).

Results - Final Figures

At the time of writing in December 1961, 20 patients have been selected for treatment. Fifteen have delivered live healthy babies, 3 are still pregnant and well past their previous abortion times, being at 35, 25 and 23 weeks maturity now, and 2 aborted exactly as they had done before at 13 and 11 weeks. This means that 90% of the patients treated have successfully avoided the abortion risk and have had or are expected to have, normal babies.

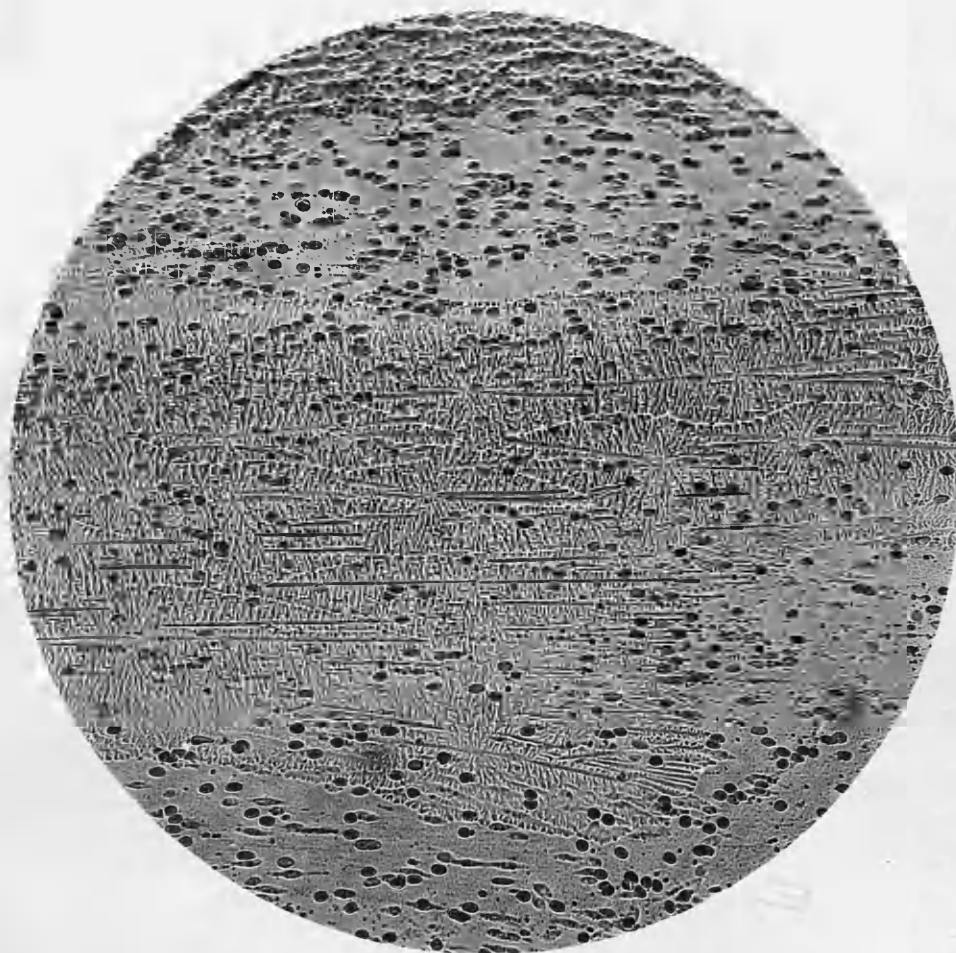


FIGURE 28.

X 140

Case 61. Two previous abortions. Moderate ferning at 6 weeks.
Progesterone therapy and normal pregnancy
but persistent crystallization.

Patients with recurrent ferning had been found to have only a 50% chance of achieving a normal pregnancy. Those with the additional problem of a previous bad history were considered to be the more difficult group with prospects considerably less than 50%. Since the patients in this group were the very ones chosen for treatment, the success rate of 90% is, therefore, all the more remarkable.

Literature and Discussion

That the choice of progesterone was fortunate as well as rational has emerged in recent years, since hydroxy-progesterone caproate is apparently the only potent progestogen without any virilising effect on the female foetus (Annotation Brit.Med.J.1961). No case of virilisation has been reported after its use, and there were no cases in the present series.

The work of Greenblatt (1956) and Davis and Weid (1957) showed that the amounts of progesterone produced naturally and required therapeutically, were considerably in excess of the quantities previously imagined. Approximately 200 mg. of progesterone are produced every day in pregnancy and unsuccessful progesterone therapy for abortion in the past may have been due to the homeopathic amounts of progesterone used, as well as to poor selection of cases.

In a study very similar to the present one, Jacobson (1960) treated patients on the same criteria of positive smears and a bad history. Sixty-one patients were given progesterone orally, intramuscularly in oil, or intramuscularly as hydroxy-progesterone caproate

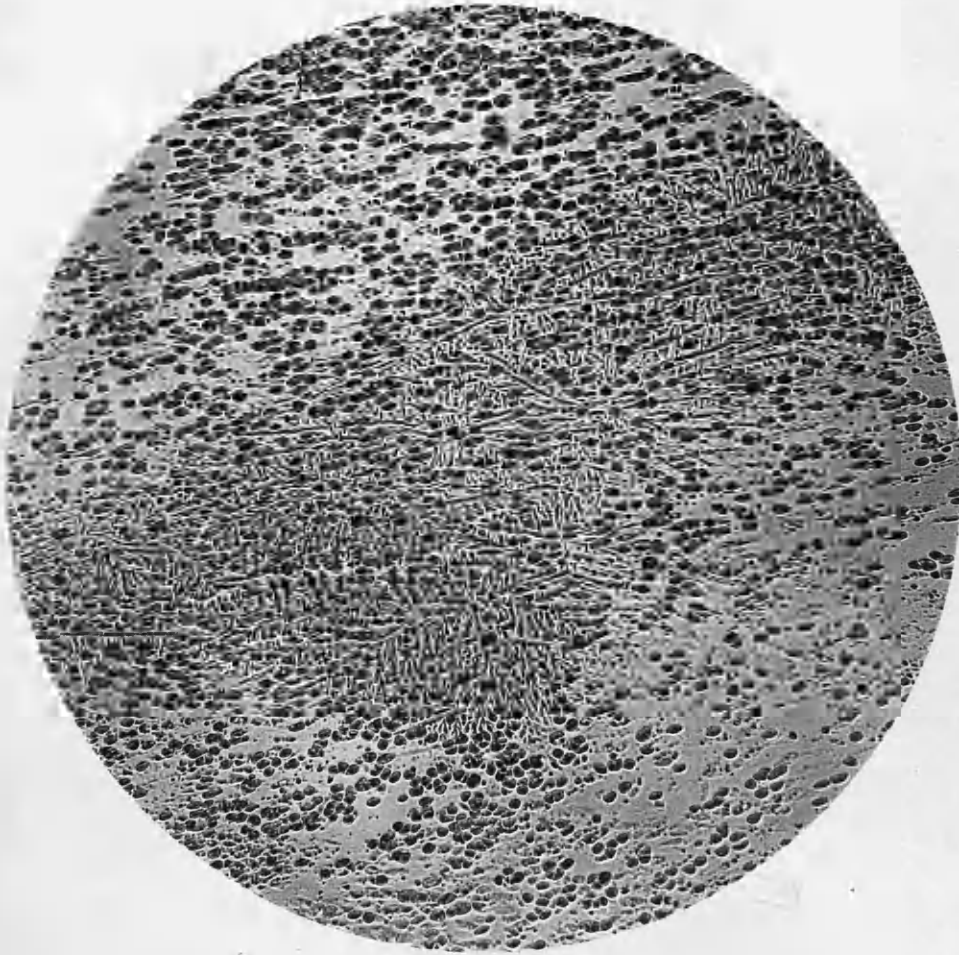


FIGURE 29

X 140

Case 60. Previous abortion. Moderate ferning.
Progesterone therapy and successful pregnancy.

together with vitamin C. Corrected results were given and cases excluded if treatment was started too late or considered to be inadequate, but the paper is credible and well presented, and he claimed a success rate of over 90%. Of the 10 patients given hydroxy-progesterone caproate, only one with a bicornuate uterus aborted. Even more interesting were 393 patients with ferning smears but good histories. None of these patients was given any treatment and all of them went to term uneventfully: 107 showed persistent crystallising smears, but in 286 the crystals cleared spontaneously from the mucus before 28 weeks.

It is significant that Case 61 with persistent ferning was the only patient under treatment who had repeated threatened abortions, confirming the association between crystals in the mucus and a tendency to bleed. Failure to utilise the progesterone might explain the lack of response. This raises again the question of the basic hormone defect and suggests that it is not a simple progesterone deficiency. Since it tends to be recurrent from one pregnancy to another, it may be a fundamental pituitary imbalance, or some failure in the progesterone metabolic chain. If this were true, then the precise form of progestational agent required might vary from one patient to another.

The explanation for the way in which some patients correct the hormone imbalance spontaneously while others require treatment to prevent abortion is not clear. The results however, suggest that the method of selection for treatment is sound.

Conclusions

1. Progesterone therapy is very effective in preventing abortion among patients with hormone imbalance characterised by salt crystals in the cervical mucus.
2. Patients with salt crystals in the mucus but with a previous good history do not require treatment.
3. 17-alpha-hydroxy-progesterone caproate is a satisfactory progestational agent and shows no evidence of any virilising effect on the female foetus.
4. Contrary to expectation, third stage complications are not more common among patients with crystallising mucus.

Summary

Twenty patients who were considered particularly liable to abort because of recurrent crystallising mucous smears and a previous bad history, were treated with progesterone. Details were given of several of the patients who went to term and also of the two patients who aborted. An overall success rate of 90% was achieved.

17 a-hydroxy-progesterone caproate was chosen as the progestational agent and the reasons were discussed.

Urinary pregnanediol estimations were carried out in several cases but the results were generally disappointing: cervical mucous crystals gave a better indication of prognosis.

It was shown that patients with recurrent crystallising smears but a previous good history did not require treatment.

C H A P T E R VI

THE VALUE OF CERVICAL MUCUS IN CLINICAL PRACTICE

This section is intended as a summary of the aims and the conclusions of the whole thesis. The work was begun out of interest in the phenomenon of salt crystallization in cervical mucus, but it has proved to be of real clinical value. Much information can be gained from the cervical mucus, and the various ways in which it can be put to practical use are described below.

The outstanding virtue of cervical mucus in clinical practice lies in the simplicity with which samples can be obtained, smears made and the results interpreted. This makes it very suitable for general use as a rapid, inexpensive and informative test of hormone function.

The quantity, appearance and viscosity of the mucus varies throughout the menstrual cycle in direct relationship to the amount of oestrogen and progesterone being produced. Salt crystals appear in the dried cervical mucous smear in the first half of the normal menstrual cycle in proportion to the oestrogen level and in the second half of the cycle, the salt crystals are replaced by endocervical cells, when the oestrogen progesterone balance is normal. The post menopausal smear shows neither salt crystals nor endocervical cells. In this way the gross characteristics of the mucus and the presence or absence of salt crystals give quite an accurate estimate not only of oestrogen production and oestrogen progesterone balance,

but also of ovarian function.

In practice two smears only are required during the menstrual cycle, one about ovulation time and one pre-menstrually to confirm normal function. When a mid-cycle smear with profuse, clear, elastic mucus and complete crystallization is followed by a pre-menstrual smear with scanty, cloudy, sticky mucus and no crystals, then it can be assumed:-

1. That there is normal ovarian function and oestrogen production.
2. That ovulation has occurred and that there is a normal oestrogen progesterone balance.

This is very useful in the study of patients with endocrine dysfunction and in the investigation of infertility.

In normal pregnancy the mucous smears resemble closely the pre-menstrual picture with scanty, cloudy, sticky material and abundant cells but no salt crystals. In the diagnosis of pregnancy, examination of the mucus after an injection of oestrogen provides a useful alternative or additional test to the gonadotrophin tests. By this method pregnancy can be diagnosed immediately after a missed period whenever this is important, and quite frequently a categorical diagnosis of non-pregnancy can be made from a single mucous smear, since the profuse heavily crystallising smear often seen with fibroids, metropathia haemorrhagica, or stress amenorrhoea, cannot occur in pregnancy.

The outstanding discovery of this work was the observation that patients with some salt crystals in the mucus in early pregnancy are very liable to abort. The presence of salt crystals in pregnancy is indicative of hormone imbalance but the precise nature of the imbalance and the source of the deficiency remains uncertain. There is, however, no doubt at all that examination of cervical mucus is the best known method of selecting patients who are likely to abort because of hormone deficiency.

In practice this information can be useful in several ways. Recurrent, non-crystallising smears indicate a normal pregnancy with less than the average risk of abortion. Disappearance of the crystals spontaneously or with treatment, indicates that the imbalance has been corrected and the risk of abortion reduced. Thus normal patients can be reassured and when treatment is given the effect can be readily assessed.

Pre-menstrual crystallization in the mucus indicates progesterone deficiency. By analogy salt crystals in early pregnancy suggested an oestrogen progesterone imbalance with a relative progesterone deficiency, and so it seemed reasonable to try these patients with progesterone therapy. Injections of high potency progesterone were given to patients who were thought to be almost certain to abort because of recurrent crystallising smears and a previous bad history, and so far a 90% success rate has been obtained. It is unlikely that simple progesterone therapy is the final answer for these patients, but the method of selection is good and the results so far

are very encouraging.

There is still more to be learned by continuing with this work. The impression was formed at an early stage that persistent crystallization in the post-menopausal patient might have some bearing on the development of carcinoma and this must be followed. In early pregnancy, detailed blood and urinary hormone studies related to the mucus findings would give a much clearer picture of the imbalance and deficiencies present and provide a more rational basis for therapy. In late pregnancy, mucous smears should help with the problems of estimating placental function and the factors which lead up to the onset of labour.

Undoubtedly there is a wealth of information to be gained simply by examining the cervical mucus.

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