OBSERVATIONS ON URINARY SEDIMENT CELL COUNTS IN WOMEN

By

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It has been repeatedly observed that the vaginal epithelium in woman responds to stimulation by ovarian hormones with cyclical proliferation and desquamation. This effect can also be seen in the urinary sediment smear cell count, as was demonstrated by Del Castillo et al. (1946, 1948, 1949), who also showed that such smears present variations parallel with those found in vaginal smears made at the same time.

For the last three years we have been using the urinary sediment smear cell count in the functional examination of our patients with such good results that we have been induced to undertake a series of studies with the object of extending the knowledge of this method, which is so simple and yet yields such useful information.

In this paper we refer to our results based on observations on 1019 smears, but have omitted to go into theoretical details which we have summarized elsewhere recently (Lencioni, 1952 a & b).

»PARALLELISM« BETWEEN VAGINAL AND URINARY SEDIMENT SMEARS

In our first studies (Lencioni, 1952 c; 1953 a) we proposed to determine by means of a strict statistical analysis the exact mathematical co-relation of the »close parallelism« which Del Castillo, Argonz & Galli Mainini (1946) referred to in their original work.

From these studies the following facts can be deduced:

a) There was a parallelism between the variations of the superficial acido-

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Phil cells1 of vaginal and urinary sediment smears simultaneously prepared and examined at the same time. The average for vaginal smears was 24.9% and for urinary sediment smears 17.5%, with a significant average difference of 7.4 ± 2.07 standard error.

b) This relationship occurred independently of the percentage of superficial acidophil cells.

c) The sensitivity of both methods was the same.

d) Two regression formulae were elaborated which allowed of the values obtained by vaginal smears to be converted to those that would be obtained by urinary sediment smears, and vice-versa. These formulae are:

\[ \hat{x} = 6 + 1.08 \times Y \]
\[ \hat{y} = 3.854 + 0.548 \times X \]

respectively.

We will clarify this statement by the following example: Suppose we wish to ascertain what percentage in the urinary sediment smear will correspond to a reading of 20% superficial acidophil cells in the vaginal smears, by using the appropriate formulae we get:

\[ \hat{y} = 3.854 + 0.548 \times X \]
\[ \hat{y} = 3.854 + 0.548 \times 20 \]
\[ \hat{y} = 3.854 + 10.96 \]
\[ \hat{y} = 14.8 \]

that is to say, for a reading of 20% superficial acidophil cells obtained in the vaginal smear the corresponding percentage will be 14.8% in the urinary sediment smear. This can also be represented graphically using the diagram in Fig. 1 which we have drawn from our data. We must add that neither of these methods can be used for the conversion of values below 10% of acidophil superficial cells.

e) Mack's (1943) staining method with iodine vapors (Lencioni, 1952 a, 1954) and in some cases Best's Carmine technique (Levinson & Mac Fate, 1946) was used to study the glycogen variations in the urinary sediment smear. From these examinations the following conclusions can be made:

1) The concentrations of glycogen in the urinary sediment smears and the vaginal smears obtained at the same time are very similar, whatever the oestrogenic level and the time elapsed (1, 5, 25 days) after extraction and staining.

1. The classification used in this work is the one suggested by Di Paola (1949), based exclusively on the staining affinity without reference to the cornification process, the nature of which is a debatable matter. Di Paola divided the cells observed in the vaginal smear into: superficial acidophils, superficial basophils, intermediate basophils, deep basophils and deep acidophils.
2) The variations in the glycogen content of the urinary sediment smear, as well as in that of the vaginal smear, cannot be used for the determination of small differences in the oestrogenic level which can be determined by other methods (Shorr, 1941; Papanicolaou, 1948; Kiserud, 1952). They are only useful in cases of marked ovarian insufficiency.

This again shows that both epithelia have similar reactions.

METHODS

There are two disadvantages usually encountered in the performance of a urinary sediment smear cell count: 1) Absence or scarcity of material and 2) a false acidophilia due to bad fixation and staining. In the following it is shown how these difficulties have been overcome (Lencioni, to be published).

a) Collection of material:

The first urine voided in the morning must be used. It is best to have about 150 ml. except when centrifugating is used when much less urine is necessary, and it can be voided at any time during the day.

b) Extraction:

Filtration: This is done by the original technique described by Del Castillo, Argonz & Galli Mainini (1946) which consists in passing the first urine voided in the morning through a filter paper, scraping the material which remains on the paper with a platinum loop and smearing it on to a slide (Fig. 2).

Aspiration: The apparatus described by Galli Mainini (1952) is used. It consists of a glass tube one end of which is joined by a rubber tube to a 20 ml. syringe.
Apparatus employed for the extraction of the material.

(or to a vacuum pump which we use) while the other end is attached to another thicker rubber tube in which a pellet of cotton wool is placed. The end of this is introduced as far as the bottom of the bottle containing the urine and about 100 ml. are aspirated. The material which is retained in the cotton wool is smeared on a slide (Fig. 2).

Sedimentation: Ferrer (1948) proposed the use of a sedimentation capsule on which a coverslip is placed (Fig. 2). This is introduced into a funnel provided with filter paper and the material leaves a sediment on the coverslip.

Centrifugation: We centrifuge for 5 minutes at 800 revolutions per minute and then make a smear on a slide.

The comparative study of these various techniques has allowed us to draw the following conclusions:

A) By using the centrifuge much more material can be obtained.

B) Filtration is the simplest procedure and specially recommended when no centrifuge is at hand.

C) The sedimentation method is probably the one in which there is the least alteration of the cellular grouping, a significant factor in its value for the study of the different phases of the sexual cycle.

c) Fixation:

Two techniques have been used:

1) The usual fixation with alcohol-ether, there being no disadvantage when strict attention is paid to all details of the technique.

2) Using dried material subjected to the following technique: The smear is allowed to dry spontaneously. To hydrate the smear it is submerged in tap water for two minutes, then passed through alcohol 30° and 70° with 2% acetic acid, 2 minutes each, and stained with Shorr's or Papanicolaou's stain. By this mean
the cells regain their staining properties. This last procedure has the following advantages:

1) The material is not submerged in a fluid of similar density thus avoiding loss; hence preparations treated in this way show more cells.

2) We have demonstrated mathematically that no modifications of the percentage of superficial acidophil cells are brought about by this technique.

3) Dried material can be easily sent to distant diagnostic centres, a considerable advantage for a wider study of the hormonal sexual functions in women and the early diagnosis of genital cancer.

d) Staining:
   1) Shorr's (1941) and Papanicolaou's (1948) stains have been used both for fixed and dry material or with the modification described by one of us (Lencioni, 1952 d; Lencioni et al., 1952).
   2) Kiserud and his associates' stains (1952) have also been used. Statistical analysis has shown that the figures obtained for the superficial acidophil cells in both vaginal and urinary sediment smear cell counts (Lencioni et al., 1952; Lencioni, 1953 b) are the same as those obtained with Shorr's stain in fixed and dry preparations. The Kiserud stains have the advantage of giving more stable solutions with firmer and brighter colours, while their price is more economical.

c) Reading of preparations:
   At least 400 cells in 7 different fields are counted. According to Allende & Orias (1947) this avoids errors due to chance accumulation of one type of cell in a given field.

THE URINARY SEDIMENT CELL COUNT AND PREGNANCY

As regards the usefulness of the urinary sediment cell count in the determination of the hormonal state during gestation we have observed the following facts:

a) In normal conditions, as is found with the vaginal smear cell count, the percentage of superficial acidophil cells does not rise above 10 % (Lencioni, to be published; Pundel & Van Meensel, 1952).

b) When there is hormonal imbalance, which in our experience coincides with a low pregnanediol excretion, there is a significant increase in the number of superficial acidophil cells.

c) In an appreciable number of normal pregnancies, as has been noted by Sakari & Soiva, 1950), the urinary sediment cell count gives exact information which is not obtained from the vaginal smear cell count in which high values for the superficial acidophil cells are found. As is well known, this false acido-
philia is easily explained if certain local factors pertaining to this condition are taken into account e. g. the frequent infection.

DISCUSSION

From the above mentioned facts it can be concluded that the urinary sediment cell count is at least as useful as the vaginal smear cell count in the study of the oestrogenic condition of the non-pregnant woman, whilst it is particularly reliable during pregnancy.

This technique is specially indicated in the following cases:

1) In girls, virgins and in special psychical conditions which make vaginal manipulations undesirable.

2) Where there is local infection or hemorrhage which falsifies the data given by vaginal smear cell counts.

3) When repeated readings are required, since this method is much less disturbing to the patient.

We suggest that the best technique, which gives the largest amount of material, is that of centrifugation and drying and staining with Shorr's or Kiserud's stains.

SUMMARY

1) Close parallelism between the vaginal and urinary sediment cell counts has been observed in a study of 1019 smears.

2) The advantages of different methods of collecting, fixing and staining material have been discussed. Centrifugation, drying and staining with Kiserud's stains gives excellent results.

3) The use of the urinary sediment cell count in the control of normal and abnormal pregnancy is demonstrated.

REFERENCES


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