CERVICAL CYTOLOGY TESTS IN CANCER DIAGNOSIS: GLYCERINE TECHNIQUE FOR MAILING*

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The vaginal and cervical cytology smear is a relatively recent development in uterine cancer diagnosis. It is rapidly becoming established as a reliable and simple method of making a presumptive diagnosis of malignancy arising from the uterus. The original technique of preparing the smears was outlined clearly by Papanicolaou and Traut.[1] In the past five years various investigators (Meigs,[2] Ayre[3]) have reported large series of cases in confirmation of Papanicolaou's[4] excellent work, in which the cytology diagnosis showed less than a 10% deviation from the pathological diagnosis.

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Papanicolaou and Traut advised taking the smear with a pipette, spreading it on a clean dry slide which was promptly immersed in a solution of equal parts of 95% alcohol and ether, and the slides were kept moist in this same solution until they were stained and mounted for interpretation. This method of fixation is quite satisfactory for hospital use where the smear is taken in the wards and then sent to the cytology laboratory adjacent, for staining and interpretation. However, when the smears are taken in an office or at any point distant from the laboratory, transportation becomes a technical problem. Bottles large enough to carry the glass slides immersed in the alcohol and ether mixture are bulky and can not readily be mailed. This difficulty has greatly limited the scope of adaptability of the smear method to general use.

Until recently it was our practice to follow this same technique, carrying the bulky bottles back and forth between office and laboratory. In an effort to evolve a technique where the cytological material could be transported by mail, the centrifuge[5] technique was worked out in our laboratory. This method consisted in aspirating the secretions from the cervix and expressing them into a test tube containing a fixative (formalin, or ether and alcohol) the test tube being mailed in a container similar to that used for Wassermann tubes. Arriving in the laboratory the cellular material was first centrifuged and then mounted in a paraffin block and sectioned similar to any tissue biopsy. This technique is particularly adaptable where the available equipment is that of a pathological laboratory. This test will tell if well-established cancer is present, but it fails to preserve the more delicate morphological and staining signs which we are beginning to recognize as pre-cancerous.

More recently we have found that it is not necessary to leave the cytology smears immersed in the fixative solution until they are stained. Dry smears were studied but were found too variable in cell definition. To preserve nuclear definition adequately to permit consistent accuracy in cancer diagnosis we have developed a new but simple technique which will permit mailing of the slides. The slides are first fixed in the usual fashion, then are removed from the fixative and a drop of glycerine is placed over the smear area, then a second slide applied to the face of the smear. As the glycerine spreads between the two slides it effectively seals over the smear, preserving the moist ether-alcohol environment of the

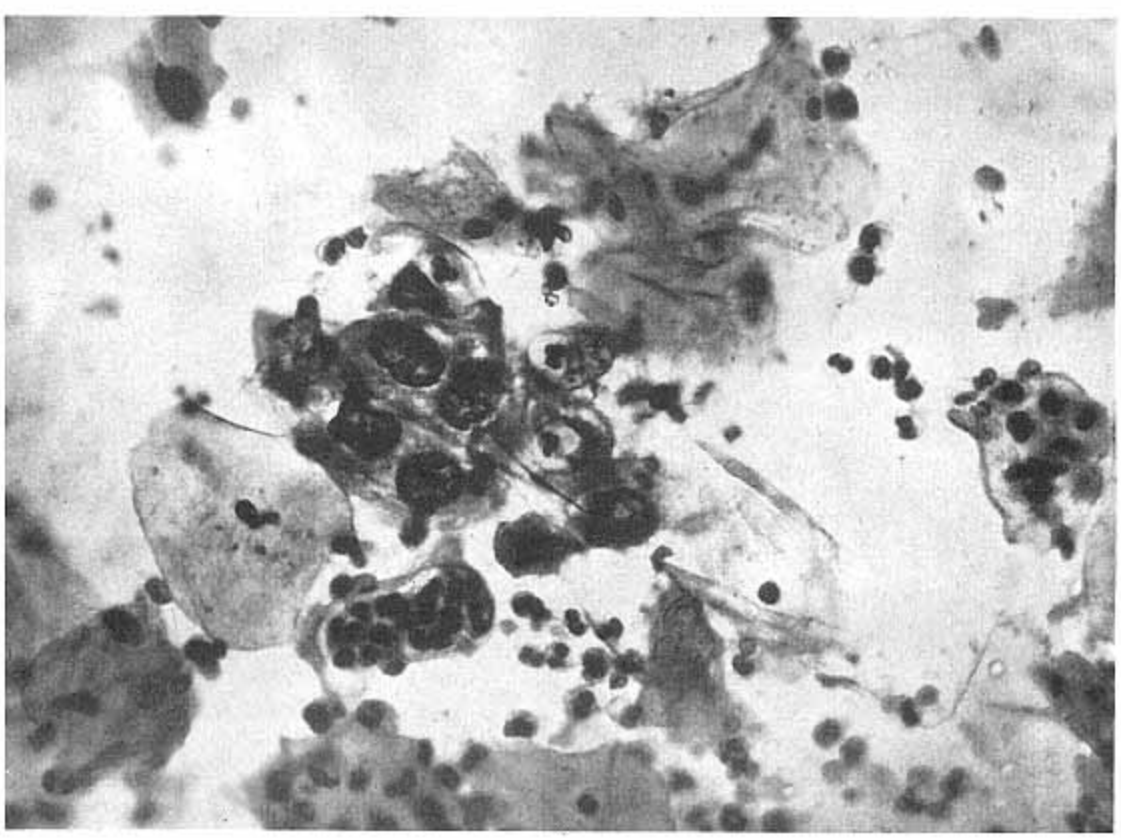

Fig. 1.—Cervical cytology smear (glycerine technique) in case of squamous carcinoma of cervix. These slides were mailed in an envelope, remaining out of solution for eight days prior to staining! Observe perfect preservation of detail of cancer cells and normal cornified elements. This is important, as recognition of abnormally high cornification (endogenous œstrogen) is a new diagnostic criterion in uterine cancer.

smear zone. An elastic placed around the slides holds them together, and they may be placed in a tiny wooden container and mailed to the laboratory for staining.

Instructions for Glycerine Technique

1. *Taking the test.*—The cervical cytology test is taken when the patient is first examined gynæcologically. Preceding bimanual examination a bi-valve speculum is inserted into the vagina without any lubricant, except possibly a small quantity applied just at the introitus. Too much of the lubricant may cause interference with the staining reaction. Following adequate exposure of the cervix, a glass pipette is used to aspirate the secretions from the external cervical os. These are transferred to one or two glass slides. The secretion is spread out over a three-quarter inch diameter area at one end of the slide. The smear should not be too thick nor too thin. As there is frequently a thick blob of mucus in the cervix, this is spread out over the slide so that the peripheral parts of the smear will be thin. Although this tenacious mucus is somewhat difficult to handle, its preservation is important as it frequently holds the exfoliated endometrial or cervical cells. Following preparation of the smear or spread, it is immediately immersed in the solution containing equal parts of 95% alcohol and ether. The slides are then left immersed in this solution for one hour.

2. *Mounting with glycerine.*—The smears may be temporarily mounted with glycerine to facilitate mailing. This is done as follows:

After standing in a fixative for one hour the slides are removed by the office or clinic technician, and without permitting drying, a large drop of glycerine is placed in the centre of the secretion zone. A second glass slide is placed face to face with the smear and the glycerine spreads out to cover the entire smear area, sealing it off completely. The two slides are then placed in a tiny wooden container in which form mailing is facilitated. The slides may remain in the temporary glycerine mounting up to two weeks if necessary, but the best staining results will be obtained if this does not exceed one week. This permits ample time for mailing to any laboratory on the continent.

3. *Staining procedure.*—This is carried out in the cytology laboratory. The cover slide is removed by rotating one slide on the other slightly preceding separation. The slide containing the cell smear is then placed in absolute alcohol for five minutes to allow the glycerine to dissolve, then staining as follows:

1. Wash in 70 then 50% alcohols, then rinse well in two changes of distilled water.
2. Stain in Harris hæmatoxylin for six to ten minutes, depending on condition of stain; older stain requires more time. Solution should be changed regularly every two weeks.
3. Rinse thoroughly three to four times in 0.5% aqueous solution HCl.
4. Rinse thoroughly in running tap water (cold) ten to fifteen minutes. If time is not a factor, fifteen to twenty minutes is more satisfactory.
5. Rinse in 50%, 70%, 80%, and two changes of 95% alcohol (do not carry any water into OG-6).
6. Stain for one minute in OG-6, depending on age of stain.
7. Rinse five to ten times in each of two jars containing absolute alcohol (95%) to remove excess stain.
8. Stain in EA-50 two and one-half to three and one-half minutes.
9. Rinse five to ten times in each of three jars containing 95% alcohol. (Fresh alcohols—not ones used after OG-6.)
10. Rinse in absolute alcohol.
11. Rinse in zylol (1) and (2), allowing to stand a couple of minutes in second zylol before mounting.
12. Mount in Canada balsam or permount.

N.B.—It is very important that water does not come in contact with slides after No. 5.

Have separate bottle for each step—do not use the 70 and 50% alcohols set up for No. 2 when coming to No. 5. The same applies to other repeated solutions.

The stain which we have found most efficacious in preserving cell detail in this technique is Papanicolaou's EA-50 and OG-6.

From an experimental study of ten cancer cases, using this glycerine technique, we have been unable to detect any loss of cellular definition or detail after one to seven days. Both cancer cells and normal cornified elements retain their original morphology and staining reaction. This fact is significant, as we have recently come to recognize that the degree of cornification is an important diagnostic sign,[6] not only in full-blown cancer but more significantly in a precancerous cervical lesion.

This development means that the smear test as a method of determining early uterine cancer diagnosis is adaptable in any location. It means that smears may be taken and transported by mail in the same manner as tissue biopsy slides are mailed.

Summary

A new cytology technique has been described whereby vaginal and cervical smears may be temporarily mounted for mailing by the described glycerine technique. The cancer cells and normal cells lose none of their morphological interpretation of cancer or pre-cancer.

The cytology test for cancer becomes as readily available to the physician's use as a Wassermann test.

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References

1. Papanicolaou, G. N. and Traut, H. F.: *Am. J. Obst. & Gyn.*, 42: 193, 1941.
2. Meigs, J. V., *et al*: *Surg. Gyn. & Obst*, 77: 449, 1943.
3. Ayre, J. E.: *Canad. M. A. J.*, 51: 17, 1944.
4. Papanicolaou, G. N. and Traut, H. F.: Diagnosis of Uterine Cancer by the Vaginal Smear, Commonwealth Fund, New York, 1943.
5. Ayre, J. E. and Dakin, E.: *Canad. M. A. J.*, 53: 63, 1945.
6. Ayre, J. E. and Bauld, W. A. G.: *Science*, to be published April, 1946.

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