URINE SEDIMENT SMEARS AS A DIAGNOSTIC PROCEDURE IN CANCERS OF THE URINARY TRACT[1]

THE demonstration that cancer of the uterus undergoes constant exfoliation which renders possible its recognition in a vaginal[2, 3, 4] or endometrial[5] smear led us to investigate the possibility of using a similar method for the diagnosis of malignant tumors of other organs.

The urinary tract appeared to be most suitable for such an investigation. One would expect that in a cancerous lesion of one of the urinary organs superficial cancer cells would become exfoliated into the excretory ducts and be carried out by the urine.

The fact that cells from a cancer of the bladder have been seen in smears prepared from the vaginal fluid[6] proves that cancers of the urinary organs undergo superficial desquamation. Recently, we have been able to ascertain that such cells may also be found in smears prepared from fluid aspirated from the female urethra.[7] For this we employed a technique similar to the one used for preparing endometrial smears.[5] A fine metal cannula[8] was introduced in the urethra and the fluid was aspirated with a lock syringe. The fluid was spread on a slide and then fixed and stained in the same way as a vaginal or endometrial smear.[4, 5, 9]

This method permits the demonstration of cancer cells in lesions of the female bladder and urethra. Its use is, however, limited, as it can not be applied to other organs or in men. For this reason we began to explore the possibility of developing a satisfactory technique for demonstrating cancer cells in the sediment of centrifuged urine.

The results were very gratifying and soon our technique was crystallized as follows: Approximately 40 cc of urine were collected in a tube and mixed immediately with 10 to 20 cc of 95 per cent. alcohol. This secured a good fixation and preservation of the cellular elements. The urine was centrifuged for ten minutes at twenty thousand revolutions per minute. It is preferable to do the centrifuging without waiting too long, although a specimen may be kept for several hours, and even days, without deteriorating too much.

After centrifugation, the supernatant fluid is removed (preferably by aspiration) and the sediment is spread with a wire loop on glass slides which have been thinly filmed with albumen. The fixation of the smears is important and must be done before complete drying, in a solution of equal parts of 95 per cent. alcohol and ether. There, the slides may be kept for any length of time, although a fixation of five to ten minutes is sufficient. The staining procedure is similar to that used for vaginal smears.[9]

Catheterized urine specimens are generally preferable to voided specimens. When cancer of the kidney or ureter is suspected, it would be desirable to obtain some urine through cystoscopic catheterization of the ureter. In prostatic lesions two specimens of urine should be collected, whenever possible, the one before and the other after massaging the prostate.

The criteria upon which the diagnosis of cancer is based are much the same as those described for the vaginal smear.[4] They are chiefly modifications and abnormalities of the nucleus (enlargement, anisonucleosis, fragmentation, hyperchromatosis, granular arrangement of the chromatin, prominence of nucleoli, etc.), changes affecting the cytoplasm (basophilia, vacuolation, leucocytic infiltration, etc.), and significant deviation of cells from their normal size and form.

Thus far eighty-three cases have been investigated.[10] Of these, seventy were men and thirteen women. The results will be reported in detail in a subsequent paper. They offer conclusive evidence that in neoplasms of the bladder, prostate, kidneys and of other organs of the urinary tract, the superficial desquamation is of such a proportion as to permit a diagnosis by urine sediment smears. The type and location of the tumor are not always clear. A longer and more detailed study is necessary for the final classification of the various types of cancer cells which are encountered.

Of the eighty-three cases, twenty-seven have been reported as positive for neoplasm on the basis of smears without any knowledge of clinical findings. In twenty-four of these (88.88 per cent.) the smear diagnosis was confirmed by biopsy (21 cases) or clinically (3 cases). In the remaining three (11.12 per cent.) the final diagnosis remained obscure. There was no case in which the smear was reported as conclusive for malignancy which definitely did not have a neoplasm.

[1] This work was supported by the Commonwealth Fund of New York.

[2] G. N. Papanicolaou, *Proc.* Third Race Betterment Conference, p. 528, 1928.

[3] G. N. Papanicolaou and H. F. Traut, *Am. Jour. Obstet. and Gynec.*, 42: 2, 193–206, 1941.

[4] G. N. Papanicolaou and H. F. Traut, The Commonwealth Fund, New York, May, 1943.

[5] G. N. Papanicolaou and A. A. Marchetti, *Am. Jour. Obstet. and Gynec.*, 46: 3, 421–2, 1943.

[6] Papanicolaou's unpublished data.

[7] The first urethral smears were prepared with the cooperation of Dr. Allister M. McLellan and Dr. J. Scott Gardner of the Department of Surgery (Urology) of Cornell Medical College and the New York Hospital.

[8] W. H. Cary, *Am. Jour. Obstet. and Gynec.*, 46: 3, 422–4, 1943.

[9] G. N. Papanicolaou, SCIENCE, 95: 2469, 438–9, 1942.

[10] Most of the specimens were obtained through the courtesy of Dr. A. R. Stevens and Dr. A. L. Dean, of the Urological Departments of the New York and Memorial Hospitals.

In the group of cases where the smear was inconclusive or negative, the percentage of correct diagnosis was lower (about 60 per cent.). This might be interpreted as indicating that in some tumors the exfoliation is less pronounced, as has already been observed in tumors of the uterus.[4] When the number of exfoliated cells is small, their presence may more easily escape attention. In most instances the smear diagnosis was based on the examination of a single voided specimen of urine. It is likely that a greater accuracy would be obtained if the tests were repeated and more catheterized specimens were used.

The small number of cases examined thus far does not permit a thorough evaluation of this diagnostic method. However, we consider the results as very encouraging. It is particularly gratifying that twenty-one (88.88 per cent.) of the twenty-seven cases reported as positive were also positive from a clinical standpoint and that false positives were absent.

The cytology of the urine smear presents its own characteristics and these will require a thorough and systematic study. We intend to carry this work further and hope that at some future time we will be able to include our findings in a monograph, comparable to the one published on vaginal smears.[4] In the meantime, we feel that other investigators should have the opportunity of applying this new technique and of exploring its possibilities. This prompted us to give an account of our observations in this short preliminary report.

The great simplicity of this method, the ease of obtaining material without inconveniencing the patient and its inexpensiveness are great assets to its wider experimental or clinical application. It might prove particularly useful whenever repeated examinations are needed, either for clarifying an obscure diagnosis or following up the results of operations or treatments, as in hormonal therapy of prostatic carcinomas.[11] After further evaluation, it might seem advisable to include this test in periodic examinations such as those conducted in public health clinics for the purpose of detecting early or unsuspected neoplasms.

Despite the technical simplicity, the urine smear method of diagnosis, like the vaginal and endometrial smear methods, is rather difficult when it comes to the interpretation of the findings, and special training is required. At its present stage, it should not be considered as a final method of diagnosis. Clinical application must await further evaluation, and treatment should not be based entirely on the results of this test. A corroboration by biopsy, whenever possible, is strongly advised.

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[11] It should be noted that estrogenic treatment causes a significant change in the cellular makeup of the urine sediment, corresponding to that caused in the vaginal secretion (G. N. Papanicolaou and E. Shorr, *Am. Jour. Obstet. and Gynec.*, 31: 5, 806–34, 1936). This consists in the appearance of cells showing marked acidophilia, pyknosis of the nucleus, cytoplasmic granules and increased glycogen content. The cancer cells, when present, stand out and make a striking contrast to the normal cells. Similar findings in the vaginal smear of cervical carcinomas following estrogenic therapy have been reported previously[4].

NOTE ON THE ADSORPTION OF THROMBIN ON FIBRIN

EARLY studies on the action of thrombin and fibrinogen showed that there is a quantitative relationship between the concentration of thrombin and the amount of fibrin formed.[1,2] The data caused many to doubt Alexander Schmidt's view, which held that thrombin is an enzyme, because it was argued that if thrombin combines with fibrinogen to form fibrin, it is not an enzyme. Later the idea developed that such observations could have been explained on the basis of adsorption, and recently Wilson[3] has found that 5.1 units of thrombin disappear from solution with each mg of clotted fibrinogen. He regards this as an adsorption phenomenon.

Actually he studied only a very limited range of the variables, presumably because an adequate amount of thrombin was not available. We have extended the work and have found the relationship shown on Fig. 1.

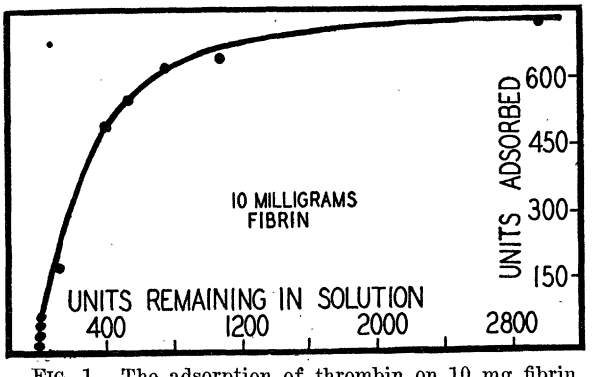

FIG. 1. The adsorption of thrombin on 10 mg fibrin, at room temperature, with a solution volume of 2 cc.

When thrombin clots fibrinogen large amounts of the former are removed from solution, and the quantity removed depends upon the original concentration of the thrombin solution. The curve showing the equilibrium relationships has the appearance of an adsorption isotherm. The new data thus support the view that thrombin is removed from solution by ad-

[1] W. H. Howell, *Am. Jour. Physiol.*, 26: 453, 1910.
[2] L. A. Rettger, *Am. Jour. Physiol.*, 24: 406, 1909.
[3] S. J. Wilson, *Arch. Int. Med.*, 69: 647, 1942.