

fame, but only to recognize it. For this they have need of observation, judgment, patience, and an open mind."

In studying the votes of the more recent elections, and particularly those cast for Gibbs through the years, it is interesting to note that he received but nine votes in 1920, the first time his name was submitted. Again, in 1935, he received 55 votes, but in 1945 the number dropped to 36. Several other candidates, like Gibbs, men of great distinction, have had comparable fluctuations in the votes by the electors through the years. As with actors on the legitimate stage and on the radio, the difficulty lies in the somewhat ephemeral character of even a great reputation. The progress of one generation sometimes obscures or outdates the achievements of a former one—a consideration that does not hold as frequently in the case of authorship or of art, where the record is more obviously and permanently made. Certainly a scientist like Gibbs, or a physician like Beaumont or McDowell, has made his record, and is it not logical to expect an organization such as the American Medical Association or some other scientific body to herald the achievements of those within its own ranks? In these days of expanded public relations and noteworthy inventions, we are under obligation to many persons for devices of large usefulness, but where the scientist or the inventor happens to be a very modest individual, as in the case of Gibbs, his work is often obscure, especially to laymen.

In the past twenty years, the public has become quite vocal in the elections, and organized groups have enthusiastically endorsed their particular candidates. The electors of the Hall of Fame are performing a national duty, and in view of this, the university has had no hesitancy in making known to the public the names and addresses of the men and women serving as electors. Many expressions of opinion concerning candidates have been sent to the electors in recent years by individuals throughout the country.

What effect these have had upon the electors, those who administer the elections of course have no means of knowing. An interesting case in point, however, was the candidacy of Thomas Paine in 1945. Through the years, Paine received the following votes: In 1920, when he was first considered, 32 votes; in 1925 his name was not on the ballot. In 1930, he was again proposed, and received 36 votes; in 1935 his popularity diminished to the point of only 15 votes; in 1940 he received 50 votes, and in 1945, the year of his election, he received 51. In 1945, while his name was being considered, a well-financed campaign was organized for his election, the details of which may be obtained by referring to a volume entitled *Public Relations in Action*, by Philip Lesly. Every device of press and radio was employed, but despite all this propaganda, he received only *one* vote more than he received in 1940. It might also be pointed out that early in 1945, before the electors voted, the three-fifths vote that was mandatory for a number of years, was changed by the senate to a majority. Had it not been for this change in the ruling, Thomas Paine, along with Walter Reed and Sidney Lanier, would not have been elected that year. These facts are mentioned to illustrate how the electors are affected by propaganda.

The university has never attempted to influence in any way the opinions of the respective electors—our position being strictly neutral. The public seems to feel, however, that the electors should be apprised of the merits of a particular candidate, and, since we are a national institution, we feel that all citizens must have an opportunity to be heard. Like a good jury, the electors of the Hall of Fame have consistently performed a difficult role judiciously. They must be of the opinion of Dr. van Dyke, who concluded his address on "Fame" with these words: "May no names be written on these walls [The Hall of Fame] except those that are lovely and of good report—names of men and women who have served humanity in their day and generation and deserved well of the Republic."



Technical Papers

A Simplified Silver Impregnation Method for Vaginal Smears¹

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The vaginal smear method for the diagnosis of carcinoma of the uterus was introduced by Papanicolaou and Traut in 1943 (1). Since that time many published reports (2) indicate the usefulness of this method as an aid in the detection of uterine cancer.

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The almost universal adoption of the stains and method so carefully developed by Papanicolaou (3) has undoubtedly resulted from the desire of most workers to apply the originally described diagnostic criteria for malignancy. This adherence to the original staining method has permitted a standardization of technique and facilitated teaching of the cytological method, but it has tended to discourage the application of other histological methods.

Recognizing that the most important cytological criteria for malignancy were based upon nuclear characteristics, the authors concentrated their efforts upon the development of a technique that would readily expose the chromatin elements of the nucleus. A pre-

liminary description of a silver impregnation method was reported by this laboratory in 1948 (4). This method was based on the more complicated diammine silver carbonate reticulum method of del Rio Hortega. Subsequently, the technical details have been improved and standardized as outlined below.

Preparation of vaginal and cervical smears: Using a saline-moistened cotton applicator, a sampling of exfoliated cells is obtained from (1) the posterior fornix of the vagina and (2) the cervical os at the squamocolumnar junction. The smear is made by rolling the applicator over the surface of a clean microscopic slide.

Fixation of smears: The smear, while still moist, is immersed in 20% formalin (1 volume formaldehyde diluted with 4 volumes distilled water) for at least 15 min. The smears may be allowed to dry following fixation or carried directly to the next solution.

Staining procedure:

1. Rinse smears in ammonia water (5-6 drops ammonium hydroxide in 100 ml distilled water).

2. Rinse successively in 2 jars of distilled water.

3. Impregnate with a medium strength silver carbonate solution for 2-3 min. This dilution of silver carbonate is prepared by adding 1 volume of stock solution to 1 volume of distilled water. The stock solution is prepared as follows: To 100 ml 10% silver nitrate add 300 ml 5% sodium carbonate. Then carefully add ammonium hydroxide drop by drop (about 10 ml) with constant shaking until the precipitate is just dissolved. An excess of ammonium hydroxide will reduce impregnation and should therefore be avoided. This solution should be stored in a brown glass bottle.

4. Remove excess silver carbonate solution by touching edge of slide to absorbent paper and reduce in 1% formalin (1 ml 40% formaldehyde diluted with 39 ml distilled water) for 1 min. Although not a necessary part of the procedure, a lighter background and one with few particles of reduced silver will be obtained if the formalin solutions for fixation and reduction are buffered to a pH of approximately 7. The authors use one of the phosphate mixtures tabulated by Hawk, Oser, and Summerson (5) to dilute Merck's reagent quality formaldehyde.

5. Rinse thoroughly in distilled water and examine under the microscope for staining quality. In the event that a heavier impregnation is desired, cover the slide again with silver carbonate solution for 2-3 sec, reduce once more in 1% formalin, and rinse in distilled water.

6. Dry in air or by rinsing successively in 90% and absolute alcohol, rinse in xylol, and mount in clarite or Canada balsam.

In this laboratory the routine evaluation of more than 3,000 cervical and vaginal smears has been accomplished without clearing and mounting (Step 6). These final steps in the procedure have been reserved for a few smears which proved unusually difficult to evaluate and for those preparations from which photomicrographs have been made.

The most practical method for applying the silver carbonate solution is as follows: Place slides face up

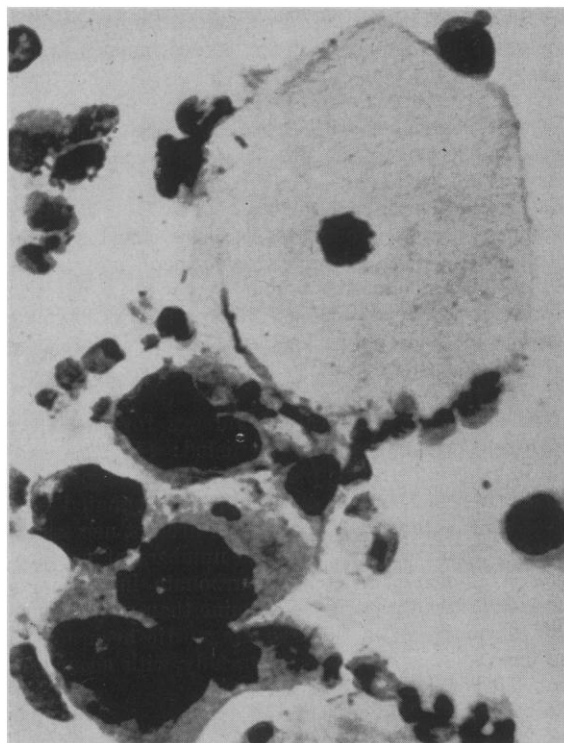


FIG. 1. Silver impregnation of a small cluster of malignant cells in a case of poorly differentiated squamous cell carcinoma. A large normal superficial squamous cell lies between the abnormal cells and a small, malignant cell. ($\times 975$.)

on parallel glass rods over any convenient collecting vessel (a commercial-type staining rack is available). Pour a small quantity of solution into a funnel with filter paper in place and successively flood each slide.

All the cellular components of the vaginal smear are readily identified following impregnation with silver. These elements may include the various types of epithelial cells from the vagina and uterus, leucocytes, vaginal flora, pathogenic organisms such as yeast mycelia, spermatozoa, and occasional erythrocytes. The red blood cells are usually hemolyzed in the aqueous formalin solution and are encountered only in preparations that have been inadvertently dried before fixation.

In general, the nuclear chromatin of epithelial cells appears intensely black following impregnation with diammine silver carbonate. The color of the cytoplasm will vary from pale yellow to dark brown. Malignant cells are usually recognized with ease because of the fact that their abnormal nuclei become deeply impregnated (Fig. 1).

Not infrequently the nuclei of normal, and especially malignant, cells are so heavily impregnated with the silver solution in the routine procedure that they appear as solid, amorphous elements. This condition obscures the details of chromatin distribution and nucleoli status within the nucleus. In any instance where a careful study of these elements is indicated, it has been found very helpful to immerse the slide briefly in a 5% solution of sodium thiosulfate follow-

ing Step 5. With a little experience, this procedure will result in a demonstration of fine chromatin detail. This step is most useful in those cases where a diagnosis rests upon the condition of the nucleoli.

From September 1947 to September 1950, this method has been applied to more than 2,000 clinical cases where there was some reason to suspect the existence of carcinoma of the uterus. The accuracy of the method was found to compare very favorably with reported accuracies for conventional, polychrome methods. These statistics will be reported in detail in another communication. Preliminary studies indicate that the method can be applied equally well to centrifuged concentrates of gastric washings and secretions of the respiratory tract.

The following technical advantages for the silver impregnation method may be listed: (1) The procedure is relatively simple, rapid, and inexpensive; (2) chromatin elements are deeply impregnated with silver carbonate; (3) counterstains are not necessary; (4) the masking effect of large numbers of erythrocytes is avoided; (5) silver carbonate impregnated smears are less fatiguing to examine than polychrome-stained smears; and (6) the diagnostic accuracy of the method compares very favorably with accuracies reported for other methods.

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The Use of Ultraviolet Light as a Means of Diagnosing Carnation Mosaic¹

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Virus diseases of the carnation (*Dianthus caryophyllus* L.) recently assumed greater importance when increased efforts were made to produce virus-free foundation stocks. However, in cleaning up these stocks the recognition of mosaic symptoms is often difficult, because of the narrow leaf in many varieties and because of complications from the effects of temperature variations and nutritional disturbances upon manifestations of symptoms.

Differential stains have been used to detect the

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TABLE 1

THE COMPARATIVE EFFECTIVENESS OF ULTRAVIOLET LIGHT AND INOCULATION OF *Dianthus barbatus* AS MEANS OF DIAGNOSING CARNATION MOSAIC

| Variety | Total no. plants tested | No. infected plants as determined by | |
|------------------------|-------------------------|--------------------------------------|--------------------------------|
| | | Ultra-violet light | <i>D. barbatus</i> inoculation |
| Scarlet King | 4,895 | 4,868 | 4,869 |
| Dark Pink Virginia | 928 | 922 | 925 |
| Hercules Virginia | 6,993 | 6,993 | 6,993 |
| Donna Lee | 6,602 | 6,593 | 6,593 |
| Pelargonium | 3,505 | 3,470 | 3,471 |
| Miller's Yellow | 2,941 | 2,939 | 2,939 |
| Northland | 578 | 577 | 577 |
| Dark Pink Patrician | 2,180 | 2,167 | 2,170 |
| Frosted Pink Patrician | 3,936 | 3,936 | 3,936 |
| White Patrician | 3,205 | 3,205 | 3,205 |
| Total | 35,763 | 35,670 | 35,678 |

presence of viruses in other plants (1-3), but they have not proved satisfactory in detecting carnation mosaic. The species *Dianthus barbatus* L. (4) has been found to be a satisfactory symptom indicator for carnation mosaic virus, but the technique of using it consumes much time and space.

MacLean and Kreutzer (5) reported that fluorescence in ultraviolet light offered a possible means for diagnosing potato virus diseases. The fluorescence of plant extract in ultraviolet light has been reported to reveal the presence of streak in carnation material (6). The same method also revealed mosaic, but it was subject to such variation that recognition was often not accurate. A modified procedure, which has proved useful in detecting mosaic virus in carnations, is reported here.

The process consists of preparing a sample of plant extract by cooking 2 g of carnation terminal vegetative shoot in .10 ml of distilled water for 45 min at 15 lb pressure.³ The cutting is then removed, and 2 ml of *n*-butanol added to the solution. The solution is shaken vigorously and allowed to settle. A drop of concentrated NH₄OH is added to the solution to increase the pH. To detect the presence of the virus, the solution should be placed before a 250-w G-E Mazda mercury-arc lamp, type A-H4, using filter No. 41,⁴ which transmits light with wavelengths between 3,253A and 4,200A. Extracts of mosaic-infected plants fluoresce a light-pink color at the interface between the water and the butanol. Solutions from virus-free plants have no similar fluorescence.

The efficiency of this technique was tested over 3 years' time by examining extracts from 35,763 cuttings from 11 carnation varieties: 4,895 Scarlet King; 928 Dark Pink Virginia; 6,602 Donna Lee;

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⁴ Manufactured by Switzer Brothers, Cleveland, Ohio.