urated with respect to its homologous enzyme system, will offer new sites for methylation to a heterologous RNA methylase. Thus a normally methylated tRNA, because of the structural configuration conferred on it by its own complement of methyl groups, may possibly expose more specific sites for methylation to a heterologous enzyme than would a tRNA whose structure is different because of the deficiency of prior methylation. The interaction of purified specific methylating enzymes with homogeneous specific tRNA's should prove to be a rewarding model for the study of the enzymatic alteration of macromolecular structure (*15*).

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Ultraviolet Absorption in Epidermoid Cancer Cells

Abstract. *The "excessive functional activity" of some cancer cells first found by Caspersson has been observed in fixed, stained smears of cervical epidermoid carcinomas from four patients. Preliminary results suggest that there may be a characteristic difference between the absorption profiles of some epidermoid cancer cells and other cells found in cytological smears. It is our belief that with an appropriate electronic scanning system such cells can be detected by measurements of their absorptions at two different wavelengths. However, the effect on the absorptions of cells with abnormalities other than cancer, and whether every epidermoid carcinoma will contain such cells, must yet be determined.*

The techniques developed by Papanicolaou (*1*) for preparing cytological smears of body surfaces or fluids are efficient for demonstrating the presence of cancer cells and are now widely used as a means of diagnosing certain cancers in an early and curable stage. It is known that use of the Papanicolaou techniques in a mass screening of the population may lead to early diagnosis of cancer of the uterine cervix with resulting reduction of morbidity and mortality (*2*). The wider application of the techniques is limited, however, by the requirement for highly trained technicians to engage in the time-consuming and tedious search for abnormal cells in each preparation. This limitation could be circumvented if a device were available for screening out a major portion of the clearly negative cases so that the cytotechnologist need only be concerned with a small fraction of the total cases.

Attempts have been made to automate the screening process based on measuring the size and visible light absorption of the nuclei of specially stained and isolated cells (*3*) and the fluorescence of cells stained with a fluorescent dyestuff (*4*). These studies have not yet resulted in a usable device. Furthermore, they require stains other than the standard Papanicolaou stain, which may be disadvantageous because they make unavailable the wealth of experience already accumulated with this stain. Thus, suspicious or problem cases must also be processed by the Papanicolaou technique for review and final evaluation. Also, many of these other preparations are not permanent and are awkward to handle on a community-wide basis.

This report is based on the work of Caspersson (*5*) and Mellors (*6*). From their work and that of others, it is now established that (i) both RNA and DNA have an absorption maximum near 2600 Å and certain proteins have an absorption maximum at 2800 Å; (ii) the average amount of DNA in the nucleus of a cancer cell, as well as the volume of the nucleus, is greater than these parameters in a normal cell; and (iii) the increased rate of protein synthesis in the cancer cell results in a greater concentration of cytoplasmic RNA than in a normal cell.

Our work with both stained and unstained material has shown that (i) absorptions with high optical contrast due to the nucleic acids and proteins of the cells were obtained even after alcohol-ether fixation and Papanicolaou staining of smears; (ii) the variability due to staining was avoided by using ultraviolet wavelengths; and (iii) particles of foreign matter, which may absorb visible light and thus become confused with cancer nuclei, did not show the specific absorptions of nucleic acids and proteins. All these facts constituted reasons for investigating the morphology of cells in Papanicolaou-stained smears at ultraviolet wavelengths. Cancer cells, as well as the various types of normal and abnormal benign cells present in a smear, were identified by well-established criteria with visible light. These same cells were then studied with ultraviolet light and relationships were established between their ultraviolet and visible light morphology. The absorption profiles of cells obtained at different wavelengths were compared to determine whether ultraviolet wavelengths offered any advantages that could be adapted to automated techniques.

Cervical and vaginal smears were fixed and stained according to the Papanicolaou technique (*1*). Quartz slides and cover slips were used. The obser-

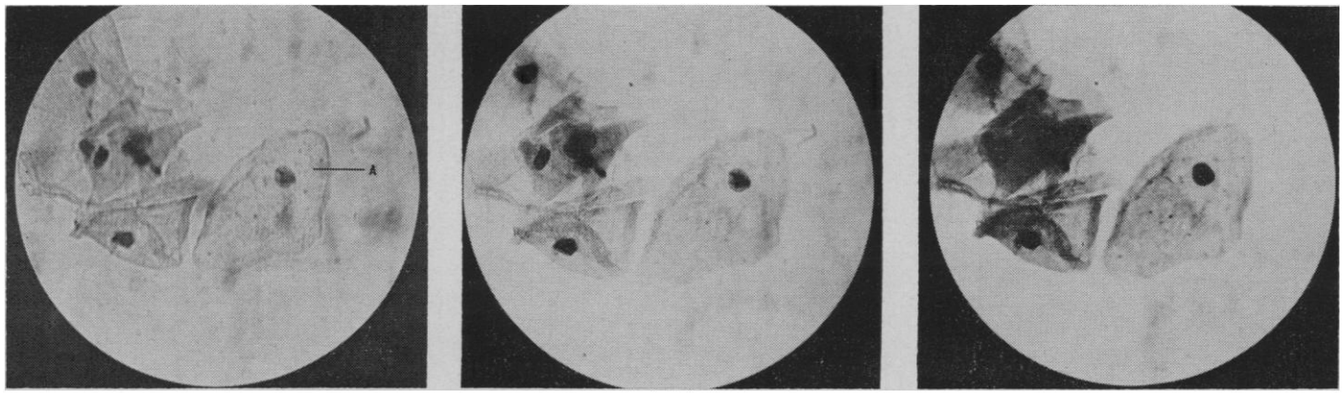

Fig. 1. Benign mature superficial squamous cells photographed at 5700 Å, 2967 Å, and 2652 Å.

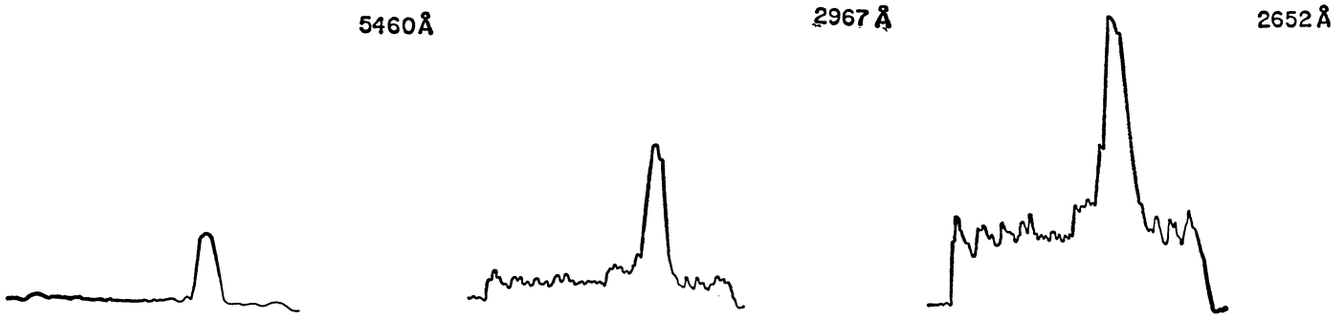

Fig. 2. The absorption profiles of cell *A* in Fig. 1 obtained at 5460 Å, 2967Å, and 2652Å.

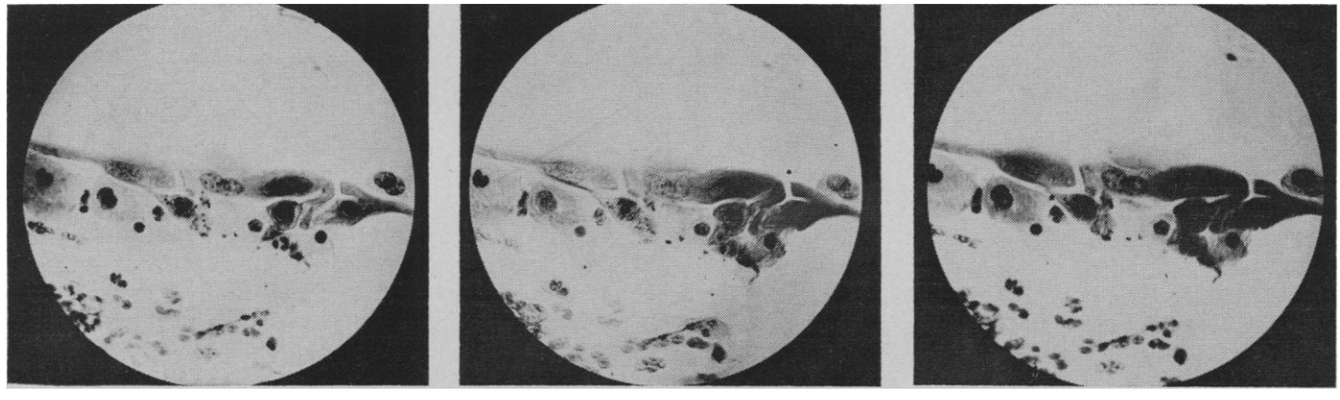

Fig. 3. Cancer cells of varying degrees of differentiation photographed at 5700 Å, 2967 Å, and 2652 Å showing the range of the total ultraviolet absorption effect.

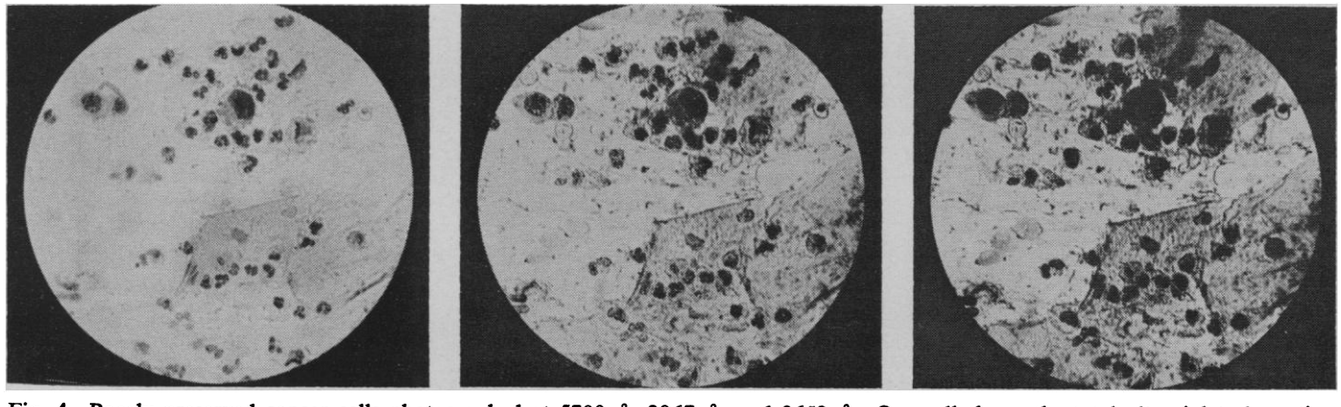

Fig. 4. Poorly preserved cancer cells photographed at 5700 Å, 2967 Å, and 2652 Å. One cell shows the total ultraviolet absorption effect.

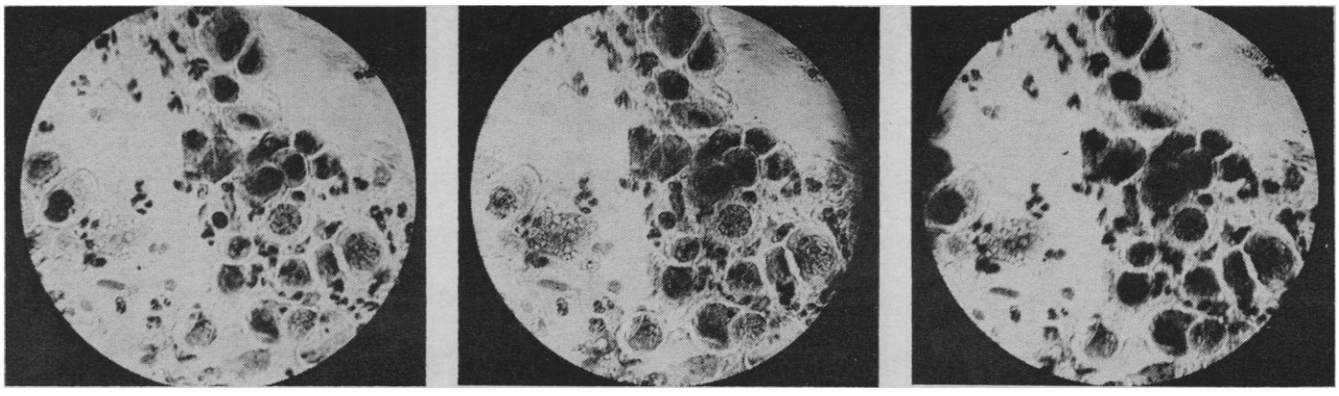

Fig. 5. Cancer cells of varying degrees of differentiation photographed at 5700 Å, 2967 Å, and 2652 Å. Some are in early stages of mitosis and each cell shows a different degree of absorption.

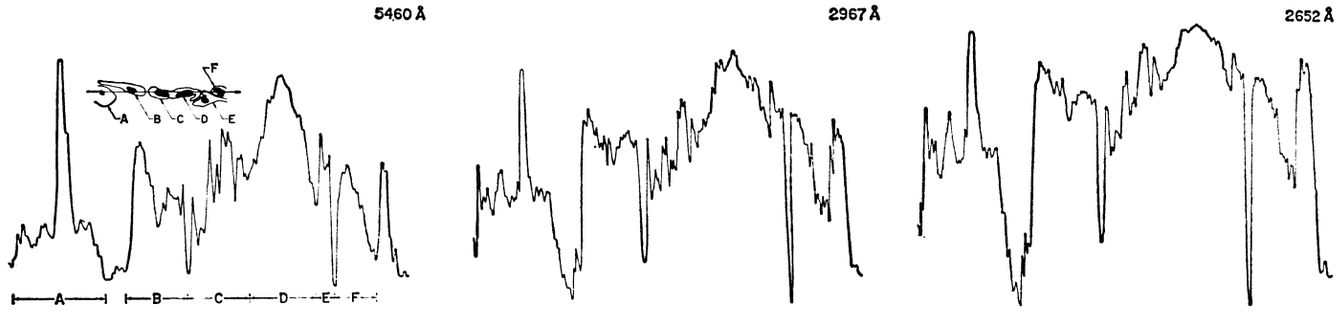

Fig. 6. The absorption profiles of specific cells of Fig. 3 obtained at 5460 Å, 2967 Å, and 2652 Å.

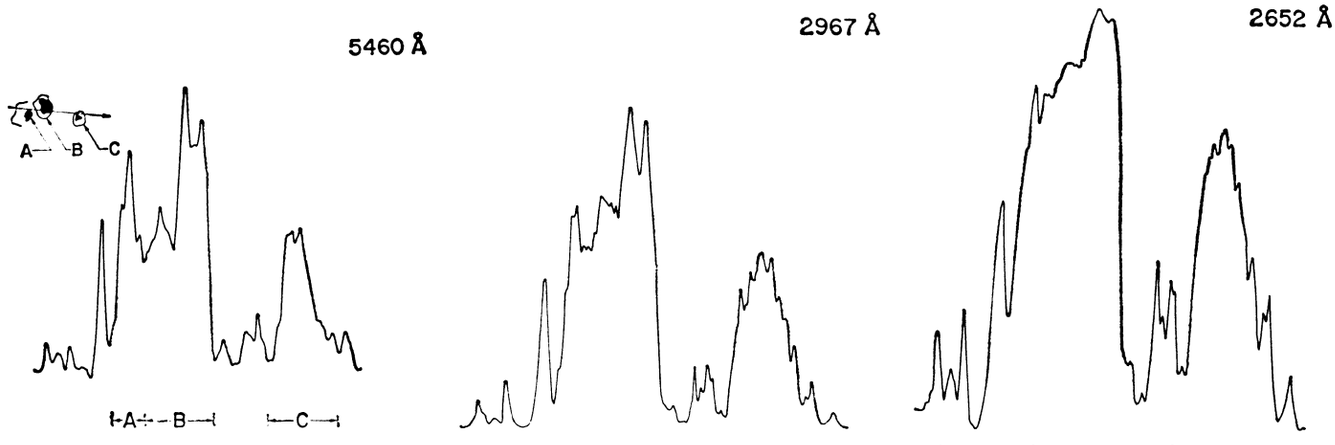

Fig. 7. The absorption profiles of specific cells of Fig. 4 obtained at 5460 Å, 2967 Å, and 2652 Å.

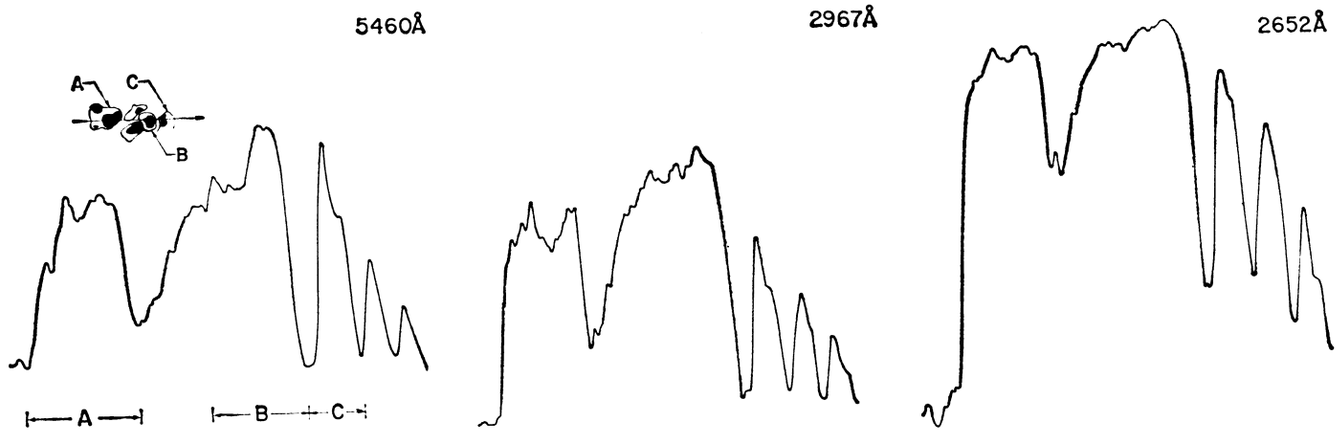

Fig. 8. The absorption profiles of specific cells of Fig. 5 obtained at 5460 Å, 2967 Å, and 2652 Å.

vations here are based on measurements of hundreds of individual cells in smears obtained from five patients. Four of these patients had histologically proven epidermoid carcinoma. The fifth patient was a normal premenopausal female whose cells were used for control and comparison purposes.

The smears were photographed at different wavelengths, with apparatus which included a Bausch and Lomb mercury light source and monochromator set to a sufficiently narrow bandwidth to isolate the 2536 Å, 2652 Å, 2803 Å, and 2967 Å mercury lines at the exit slit. A Carl Zeiss ultrafluar 100/1.25 glycerine immersion objective was used, an achromatic 0.85 NA condenser, a quartz 10× eyepiece, and a Polaroid Land camera attachment with a viewing eyepiece. Polaroid type-32 film was used at exposures ranging between 0.1 and 5 seconds.

The absorption profiles of cells were obtained by modifying the photographic equipment. The mechanical stage of the microscope was arranged to drive the slide electrically through the center of the field at the rate of 2 μ per second. An aperture and a 1P28 photomultiplier were substituted for the camera. The effective aperture size at the slide was less than 0.5 μ. The photomultiplier signal was amplified by a d-c amplifier with a bandwidth of 10 cy/sec, and the signal was recorded as a function of time on a Moseley chart recorder. The signals were normalized at each wavelength by manual adjustment of the amplifier gain while an area without cells was being scanned.

The smears were observed under visible light, and specific cells were selected to be photographed and scanned at different wavelengths. A parfocal viewing eyepiece and the achromatism of the optics enabled focus to be maintained at ultraviolet wavelengths. A scan profile line was established by correlating the aperture and the mechanical stage movement with an engraved disk in the viewing eyepiece. The reproducibility of the profiles was verified by scanning at each wavelength in succession and then repeating the measurements at all wavelengths.

Figure 1 shows photographs of typical mature squamous cells, and Fig. 2 shows the scan profile of one of these cells at wavelengths 5460 Å, 2967 Å, and 2652 Å. The nuclear boundary of these cells can be distinctly discerned at all wavelengths because of the small amount of cytoplasmic absorption.

The most striking characteristic of some of the cancer cells shown in Figs. 3, 4, and 5 is the marked absorption of the nucleus and the cytoplasm at 2652 Å due to the great amount of DNA and RNA in these cells. This often results in the disappearance of the nuclear boundary in such cells at this wavelength. The boundary is clearly present in all photographs at wavelengths above 2967 Å. In other cancer cells shown in Figs. 3 to 5, there is increased absorption of the nucleic acids but the delineation of nucleus from cytoplasm is preserved. Differences in the absorption profiles among 2652 Å and 2967 Å and visible light are shown in Figs. 6 to 8 for certain cells indicated in the figures.

Especially in Fig. 3, the absorption due to cytoplasmic RNA in some of the cancer cells is as intense as that of the nucleus, and it is not possible to distinguish the cytoplasm from the nucleus in the photograph taken at 2652 Å. It must be pointed out that in some of the other cancer cells, also present in this field, this absorption phenomenon is not nearly as complete as it is in cells *D* and *E* of Fig. 3. The inference suggested by this observation is that the cytoplasmic absorption may vary inversely with the degree of differentiation of the cancer cells. Cell *A* of Fig. 3 is an example of a well-differentiated cancer cell. In such cells the nuclear boundary is not obliterated at 2652 Å.

We believe that, with an electronic scanning system in which two wavelengths are used, cells such as *D* and *E* of Fig. 3 can be readily identified among other cells. While we have observed these cells in the four cancer cases studied, it remains to be seen by further efforts whether every epidermoid cancer will contain such cells. Special concern will be needed for some of the precancerous states in which the abundance of RNA may not equal that in cells *D* and *E* of Fig. 3, and might produce a pattern approaching that of cell *A*. It further remains to be seen to what degree actively growing or metabolizing benign cells with increased cytoplasmic proteins will exhibit the total absorption phenomenon (*7*).

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Carbamyl Phosphate Synthesis in the Earthworm *Lumbricus terrestris*

Abstract. *The enzymatic synthesis of citrulline from ammonia, bicarbonate, adenosine triphosphate, and* L-*ornithine takes place in the soluble fraction of gut tissue of the earthworm. The synthesis occurs at low ammonia concentrations, is dependent upon added* N-*acetyl-*L-*glutamate, and results in incorporation of the bicarbonate carbon into the ureido group of the citrulline molecule. Carbamyl phosphate is the intermediate in the reaction and its biosynthesis in the earthworm is mediated by a carbamyl phosphate synthetase system similar to that of ureotelic vertebrates.*

The key role of carbamyl phosphate in the *de novo* biosynthesis of arginine and uridylic acid has previously been discussed and the importance of the comparative biochemistry of this compound has been emphasized (*1–3*). Three distinct enzyme systems differing in mechanism of action are known for the synthesis of carbamyl phosphate. These are the carbamate kinase of bacteria, the carbamyl phosphate synthetase found in the fungus *Agaricus bisporus*, and the carbamyl phosphate synthetase of ureotelic vertebrates. The vertebrate system has been of interest in relation to biochemical evolution because of its role in ammonia detoxification by way of the urea cycle. The aquisition of this detoxification mechanism during evolution has been implicated as an important factor in the exploitation of the land habitat by ancestral vertebrates (*1, 2*). In the animal kingdom, studies on the synthesis and metabolism of carbamyl phosphate and the distribution of the participating enzymes have been mainly restricted to vertebrates (*1*). The utiliza-