Fluorescence microscopy in colonic exfoliative cytology

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Since the introduction of the exfoliative cytological technique in clinical practice, particularly in the diagnosis of malignant disease of the female genital tract, many authors (Friedman, 1950; Mellors and Silver, 1951; von Bertalanffy, Masin, and Masin, 1956; Liu, 1961) have endeavoured to reduce the time required for scanning the smears by the use of fluorescent microscopy. In this new technique, they have employed fluorochromes to stain the smears, and then scanned them, using an ultra-violet microscope unit. Although this technique reduces the scanning time, since the microscopist need only inspect areas on the slide which are fluorescing, recent work (Caspersson, and Santesson 1942; Bertalanffy, 1960) appears to suggest that the technique of recognizing malignant cells by demonstrating their cytochemical composition may be as reliable as the morphological criteria used by Papanicolau and Traut (1941).

A series of smears has been examined using a fluorescent microscopy technique, and acridine orange, as the fluorochrome with a view to reducing the time required to scan the material collected from the colon. The findings are briefly reported.

APPARATUS

The fluorescent microscope unit (Fig. 1) used was similar to that described by Armstrong (1957) and the smears were stained with acridine orange using the technique of von Bertalanffy et al. (1956). Some difficulty was experienced in providing adequate illumination of the smear for use with both low-power and high-power objectives, and Smiles (1958) recommended that ideally a special dark-ground condenser should be computed for this work.

FLUORESCENT MICROSCOPIC APPEARANCE OF NORMAL AND ABNORMAL EXFOLIATED COLONIC CELLS

Under the low-power objective, the cellular content of the smears appeared as numerous points of light on a dark background. Closer inspection under the low-power objective revealed that some of the fluorescing objects were cells and others were inert debris, chiefly strands of mucus (Fig. 2). With acridine orange as the fluorochrome, the ribonucleic acid (R.N.A.) in the cytoplasm and in the nucleolus fluoresced bright red, and the deoxyribonucleic


FIG. 2. A typical low-power field of fluorescing cells and mucus in a smear of material from the colon × 120.
FIG. 3. High-power photomicrograph of fluorescing normal cells. (a) A sheet of normal columnar epithelial cells × 120; (b) a single columnar epithelial cell × 480; (c) squamous cell with overlying strand of mucus × 480; (d) histiocyte with background of fluorescing bacteria × 480.
acid (D.N.A.) in the nuclear chromatin fluoresced green or greenish-yellow. The varying content of these two substances in the cells determined the degree of fluorescence and their appearance.

**SQUAMOUS EPITHELIAL CELLS** The cytoplasm of these cells (Fig. 3c) is grey with a faint greenish tinge, whereas the nucleus fluoresces green. In Fig. 3c the strand of fluorescing material running across the squame is mucin which fluoresces yellow-green.

**COLUMNAR EPITHELIAL CELLS** The feature of these cells (Figs. 3a and 3b) was the orange fluorescence of the cytoplasm. The nucleus fluoresced green and no orange fluorescence was seen within the nuclear membrane of the normal columnar epithelial cells.

**POLYMORPHONUCLEAR LEUCOCYTES** The nucleus of the cells fluoresced a bright green-white and when aggregated in groups they produced intense areas of fluorescence on a smear. The cytoplasm was grey and often difficult to distinguish.

**HISTIOCYTES** These cells were easily recognized with acridine orange staining, the foamy cytoplasm with a roughened edge fluorescing a grey-green colour (Fig. 3d). The eccentrically placed nucleus fluoresced green.

**BACTERIA AND SPORES** Bacteria fluoresce orange-red and were visible as small points of light on all the smears, as, for example, in Figure 3d. Spores fluoresced in a similar bright orange.

**FAECAL DEBRIS** Faecal material in the smears, as stained to a varying degree by the acridine orange and if present in any quantity, fluoresced a dull orange colour, presumably due to the large bacterial content.

**MALIGNANT CELLS FROM ADENOCARCINOMA OF THE LARGE BOWEL.** The nuclei of the malignant cells fluoresced a very bright yellow-green, the yellow content of the nucleus being in contrast with the nuclei of normal columnar cells. Some of the malignant nuclei in freshly stained specimens contained small areas of orange-red fluorescence. These red areas became less obvious if the specimens had been exposed to the ultra-violet light for any length of time. The cytoplasm of the malignant cells fluoresced bright orange-red, and in some cases the fluorescence had a yellowish quality which differed from the fluorescence of the cytoplasm of the

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**FIG. 4.** High-power photomicrograph of a group of exfoliated malignant cells from the large bowel × 480.

**FIG. 5.** High-power photomicrograph of a group of exfoliated malignant cells, showing nuclear budding × 480.
normal columnar epithelial cells. Apart from the
bright fluorescence of the malignant nuclei, which
together with their increased size caused them to
stand out on the black background of the smear, the
typical morphology of the malignant nuclei stood
out well. Figures 4 and 5 show groups of fluorescing
malignant cells with bright yellow-green nuclei. The
variation in shape and size of the nuclei is very
obvious, and also budding, a less common feature of
malignant nuclei, is seen in two of the nuclei. Red
blood cells were only seen if the material had been
fixed in formalin and they then showed a faint
greyish fluorescence.

CONCLUSIONS
Exfoliative cytology has proved its worth as a
method of detecting cancer of the gastro-intestinal
tract (Raskin, Kirsner, and Palmer, 1958; Oakland,
1961; Schade, 1960; Burn and Sellwood, 1962), and
it now remains to explore any method which reduces
the time required to examine the material, so that
the technique can be used to screen patients who are
suspect or at risk (Day, 1961), for example those
suffering from ulcerative colitis.

The method of collecting specimens from the
colon by saline lavage results in larger quantities of
material to process and examine than in the now
well-established gynaecological technique. Six to 10
slides are required for the material collected from
a well-prepared colon compared with the one or two
slides for gynaecological material. In this respect
there is no doubt that the staining technique using
acidine orange is quicker than the more complex
Papanicolaou technique.

There is no difficulty in setting up an adequate
ultra-violet microscope unit using a dark ground
condenser. Theoretically, acidine orange fluoresces
most brightly when excited by light energy of a
wavelength of 365 mμ (Porro, Dadik, Green, and
Morse, 1963). For practical purposes excellent
fluorescence is obtained using a Mazda mercury
vapour lamp as a light source and a single ammoniacal
copper sulphate filter to absorb the red end of the
visible spectrum. For routine scanning no advantage
has been gained by the use of transmission filters
limiting the wavelength of the light energy to the
region of 365 mμ.

The use of a microscope mounted in the horizontal
position on an optical bench is convenient for
initial work and photography, but for scanning it is
more comfortable to have the microscope in the
usual inclined vertical position and a polished
aluminium mirror to reflect the light.

Smears stained by this technique can be scanned
more quickly than by the conventional method
using the Papanicolaou stain and white light
microscopy. Most smears (5 × 2 cm.) can be
scanned in three minutes which means that all the
material from a colonic washing can be examined in
under 30 minutes.

The early hope that malignant cells would stand
out brilliantly on a dark background was not
entirely realized because of the fluorescence from
strands of mucus, bacteria, and groups of poly-
morphonuclear leucocytes. However, the exfoliated
malignant cells can be easily recognized by their
bright fluorescence and, when present, by the
particular flame-red fluorescence of the cytoplasm.
In freshly stained cells the red spots of ribonucleic
acid in the nucleoli are a particular feature of
malignant cells but these tend to fade fairly quickly.
Despite the brighter fluorescence of malignant cells,
the final classification may still depend on morpho-
logical features. These are usually easily seen, but in
the case of doubt the smear can be rapidly de-stained in
alcohol and re-stained by the Papanicolaou (1942)
method for final evaluation.

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