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Negative cytology preceding cervical cancer: causes and prevention

I read with interest the article of Robertson and Woodend.¹ The authors reviewed 140 negative smears from 103 women who subsequently developed cervical cancer ("patients with microinvasive disease were excluded"). Forty eight smears (including one smear with severe inflammatory changes) were negative for abnormal cells (34.5%). As the authors consider of importance the "assessment of the quality of the smears received by a laboratory" it is surprising to read that the causes for true false negative smears were not considered.

True false negative smears may result from a series of reasons. Some may be avoided, provided that the patient, and the persons in charge of the collection of the material and of the staining of the smears, are well aware of the pitfalls of the method. In fact, the instrument used for the collection of the smears may in itself entrap atypical cells (figure 1), thus contributing to a false negative smear,² as the atypical cells collected from the area with cervical neoplasia may never reach the slide. The *type of instrument used is also important*; a significantly lower number of atypical cells are transferred to the slides by cotton swab applicators and plastic spatulas than by cervical brushes.³⁻⁵ The method by which the smear is handled also has an important role, and variables such as (a) the technique used to deposit the material on to the slide,⁶ (b) the pressure exerted when smearing the material,⁷ and (c) the quality of the cervical

mucus⁷ may also influence the presence of atypical cells in a cervical smear. Moreover, during *staining procedures*, detached material from the slides containing abnormal cells may render smears free from atypical cells, the result being a false negative report.⁸ Also, the detached material may become attached to other slides stained in the same batch (obtained from women without cervical neoplasias). The atypical cells attached to an "innocent" smear may yield a false positive smear.⁹

One other important factor which was not mentioned by Robertson and Woodend is the patient herself. In earlier investigations we showed that smears taken immediately before conisation in cases showing histopathological dysplasia or carcinoma in situ were often negative for atypical cells.¹⁰ Shortly before taking the smear, the cervico-vaginal area had been thoroughly disinfected with a cotton swab. The vigorous rubbing of the cervix in order to disinfect the surgical area may have removed the superficial layer of atypical cells that are usually collected by conventional techniques of cell sampling. A Swedish gynaecologist found that about one third of his patients had used manual washing deep into the vagina a few hours before examination. Such a washing should have the same effect as the "exfoliation" caused by disinfection before conisation. In some countries the use of vaginal douches is widespread. Thus the patient must be instructed to come to the gynaecologist "unprepared" for a cytological test. One other common finding in smears is the presence of well preserved spermatozoa, suggesting trauma of the cervical mucosa in the hours preceding cell collection (the absence of the spermatozoa, of course, does not rule out coitus). The effect of trauma in removing superficial layers of cervical epithelium has not been sufficiently emphasised.

It is obvious that factors other than the "screening fatigue" mentioned by Robertson and Woodend may be at stake, and that all participants in a cytological examination (the patient herself, midwives, staining tech-

nicians, screeners and (alas) even doctors) should receive the proper information and education, so they are aware that each step in the procedure is important to achieve good results.

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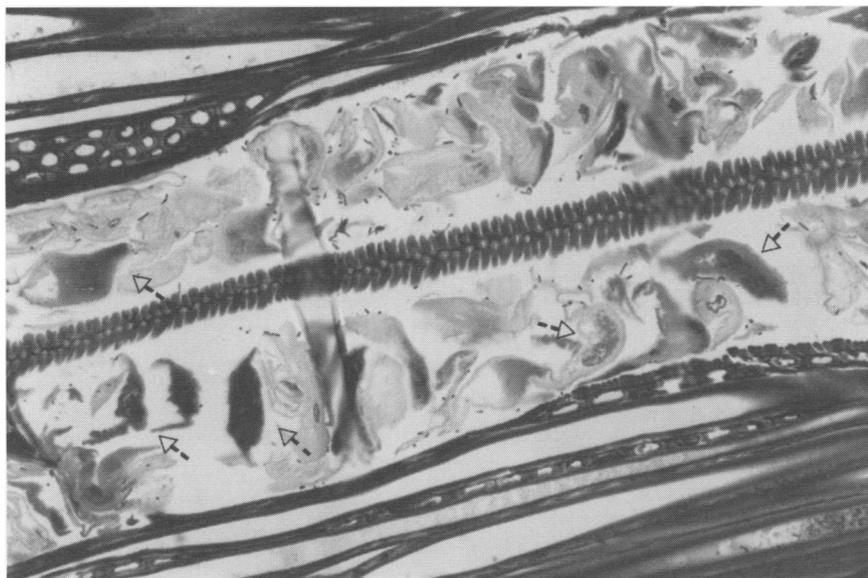
Drs Robertson and Woodend comment:

Dr Rubio draws attention to potential errors in the screening process mostly related to the taking of smears in the clinic. Our purpose was to deal mainly with the laboratory examination of smears. Dr Rubio is quite correct, however, to emphasise the importance of a good smear-taking technique and other pitfalls described in his many studies.

Cytotoxic activity of *Helicobacter pylori* enhanced by acetohydroxamic acid

One of the major pathogenicity factors described for *Helicobacter pylori* is its strong urease activity, which enables it to survive in the acid environment of the stomach. It has therefore been suggested that acetohydroxamic acid (AHA), a potent inhibitor of various bacterial ureases including *H pylori* urease,¹ be incorporated into therapeutic regimens aimed at eradicating *H pylori*. Synergistic effects between AHA and various antimicrobial agents against *H pylori* in vitro have recently been reported by Phillips *et al* in this journal, making this approach even more attractive.² As we have observed that AHA enhances the cytotoxic effects produced by *H pylori*, we would like to add a cautionary note to the discussion on the possible use of AHA for treatment of *H pylori* infection as suggested by Mooney *et al*³ and again by Munster *et al*.⁴

We have been looking at the cytotoxic effects produced by supernatant fluids of *H pylori* sonicates on HeLa cells in a quantitative microtitre cytotoxicity assay, performed in a modification of a test described by Gentry *et al*.⁵ Briefly, sterile filtered (0.45



Section of a wooden spatula after collection of cervical material before conisation for carcinoma in situ. Note a large number of normal and atypical squamous cells trapped in the wooden fibres of the spatula (toluidine blue stain).