Technical method

Application of the Papanicolaou stain to routine histological examination of placenta

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Since its introduction by Papanicolaou (1942), the Papanicolaou staining procedure has gained universal acceptance in cytological examinations. In routine vaginal cytological preparations the Papanicolaou stain demonstrates monilia species and a number of different organisms, and also provides excellent cytological staining of both nucleus and cytoplasm. Chorioamnionitis is one of the commonest abnormalities seen in placental specimens, and the organisms involved are usually those present in the vagina. It seemed logical, therefore, to use the Papanicolaou staining procedure to study placental histology.

Material and methods

In the initial stages of the study, selected cases of monilial chorioamnionitis, toxoplasmosis, cytomegalovirus placentitis, and other viral placentitides were selected. To these cases, the placentae with chorangiomas, infarcts, meconium staining, amnion nodosum, bacterial chorioamnionitis, and placentae from mothers with gestational diabetes were added.

Results

We were surprised by the quality of the results of the Papanicolaou stain. Monilia species were demonstrated as fine delicate pseudohyphae invading the tissue in a case of monilia chorioamnionitis and funisitis. Toxoplasma pseudocysts stained a distinctive orange-brown and could be separated clearly from other structures. Papanicolaou-stained sections gave excellent nuclear and cytological detail, and this enabled an extremely ready recognition of plasma cells in the chorionic villi in a case of cytomegalo-

virus infection, and when inclusion bodies were present they, too, were readily identified. Excellent histological delineation of the structure of a chorangioma was obtained and infarcts were clearly delineated by the technique. In an infant whose mother had suffered from pre-eclamptic toxæmia, evidence of atherosclerosis of the decidual basal vessels was identified fairly readily with this particular technique.

In addition to these obvious advantages of the Papanicolaou stain, most of which could be predicted, certain additional bonuses were obtained. In cases of chorioamnionitis, prominent eosinophilic degeneration of the subepithelial layers of mesenchyme below the amniotic epithelium were readily apparent; cases of vernix granuloma were easily identified, and the residual squames within the granulomata stained strongly eosinophilic. Islands of squamous metaplasia, not surprisingly, were extremely readily identifiable in varying stages of their development, and, when present, deposits of calcium stained a rather striking hue of haematoxyphilic blue.

In addition, it was apparent that the Papanicolaou acts very much like a trichrome stain in distinguishing smooth muscle cells, which stain distinctively, from the nondescript blue-green of the undifferentiated mesenchyme. This was of particular use in the study of vascular lesions occurring in the fetal chorionic circulation. Pigmented meconium-containing cells in the subamniotic mesenchyme were more readily identified by using the Papanicolaou stain, than in parallel sections stained with haematoxylin and eosin. With the Papanicolaou stain these cells, an indication of prior discharge of meconium into the amniotic space, stained dark black-brown and could be identified with ease.

Using the Papanicolaou stain differentiation of cytotrophoblast and syncytiotrophoblast cells was extremely easy. The faintly granular, pale appearance of the cytoplasm of the cytotrophoblast and open chromatin pattern of its nucleus contrast strongly with the eosinophilic cytoplasm and densely haematoxyphilic nucleus of the syncytiotrophoblast. When granulomata with Langhans type giant cells are present, as in congenital syphilis, the nuclei of the inflammatory giant cell are seen to have a distinctly different appearance from syncytiotrophoblastic cells with which they may be confused.

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Discussion

We believe that the Papanicolaou stain has certain great advantages over the routine haematoxylin-and-eosin stain preparation. Apart from the ready identification of certain categories of microorganisms in infections of the placenta and its membranes, not surprisingly, the Papanicolaou technique provides excellent cytological differentiation and enables ready recognition of many varieties of placental lesions. We now use the Papanicolaou stain routinely

for all placental histological examination and find it both aesthetically pleasing and useful. It has helped to reawaken interest in placental histology among our colleagues at different levels, and, even if it has no other merits, in our eyes this facet alone justifies its routine use.

Reference

Papanicolaou, G. N. (1942). A new procedure for staining vaginal smears. *Science*, **95**, 438-439.

Letters to the Editor

Antibiotic susceptibility testing by rotary inoculator

There is, at present, considerable interest in many aspects of pathology laboratory safety and, in particular, microbiological hazards (Collins *et al.*, 1974; Department of Health and Social Security, 1975; Harvey *et al.*, 1976). During the last two years there has been an increase in the use of the control comparison technique of antibiotic susceptibility testing described by Stokes (1975) and, in order to carry out the technique rapidly, many laboratories use a rotary inoculator and the MASTRING-S. The inoculation of the culture plate is carried out on the rotating head (Figure) (Pearson and Whitehead, 1974) by spreading a drop of the test organism suspension with a dry swab from the centre of the culture plate to the delineation point which coincides with the MASTRING-S tips. A control sensitive organism is spread with a pre-prepared swab (Felmingham and Stokes, 1972) from this point to the plate edge.

The inoculator head rotates at approximately 150 rpm and the possibility has been raised of production of aerosols during this procedure. Therefore, a test for the production of aerosols was carried out by the following simple technique.

Plates (9 cm) containing 15 ml Blood Agar Base S (Mast Laboratories Ltd) with whole blood (7%) were held at 7.5, 15, 22.5, and 30 cm distance from the centre of the rotating head, directly above it, at an angle of 45°, and vertically at the side, the face of the blood agar plate

being held towards the head. Suspensions equivalent to a Brown's No 1 of *Serratia marcescens* and a fluorescent strain of *Pseudomonas aeruginosa* were prepared. A culture plate dried for 30 minutes was placed on the rotary inoculator and seeded in the centre with a 2 mm loopful of the first suspension and a dry swab was moved from the centre to the edge of the plate. The inoculator was run and the swab was moved slowly into the centre and back to the edge of the plate twice. The time taken for this operation was approximately 20 seconds from the removal of the lid until

its final closure. This was repeated using fresh plates for both organisms, for all angles and distances. A swab was then dipped into the first suspension and soaked with it. This *wet* swab was placed directly on to a clean culture plate on the rotary inoculator and moved from the centre to the edge of the plate and returned four times with the inoculator rotating. The total times for this operation was approximately 25 seconds. This was repeated for both organisms, for all angles and distances.

After completion of these tests, a check was carried out on the ability of the test system to demonstrate aerosols. A hot loop was placed in a dish of the suspension with a plate held at 7.5 cm from the loop. It was expected that much spattering of the suspension would occur (Collins, 1976).

Using the *dry* swab, no bacterial aerosol was detected at any distance or angle. With the *soaked* swab, only on one plate at 7.5 cm and 45° were two colonies of *Ps. aeruginosa* found, and all other plates showed no growth. In contrast, when hot loops were inserted into dishes containing the suspension, culture plates held nearby showed approximately 100 colonies per plate for both organisms.

These tests show quite clearly that the rotary inoculator, at a speed of 150 rpm especially with the usual dry swab technique, produces no significant aerosol and appears to be safe. Probably the most dangerous aspect of the use of the machine is the transference of loopfuls of culture on to culture plates, and

Figure The rotary inoculator with a swab held at the delineation point.