Preliminary Communications

Histochemical Demonstration of Pentose Shunt Activity in Smears from the Uterine Cervix

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It has been shown (Chayen, 1961 ; Chayen *et al.*, 1962) that in suitably prepared specimens the response to the histochemical reaction for a dehydrogenase of the hexose-monophosphate pathway (or "pentose shunt") was more intense in cancerous cells than in normal cells. It seemed that histochemical methods for one or other of the two dehydrogenases of this pathway might be of use in "staining" malignant cells in cervical smears. Accordingly such smears have been subjected to histochemical reactions for both these enzymes—namely, the dehydrogenases which act on glucose-6-phosphate and on 6-phosphogluconate.

MATERIAL AND METHODS

Scrape smears (by the procedure described by Way, 1963) were made from the cervixes of 15 normal patients—that is, those in whom smears had revealed no abnormal cells when examined by the Papanicolaou technique—and of 15 patients who had histologically confirmed carcinoma of the cervix. In the earlier work the undried preparations were immediately flooded with the incubation medium and left in a humidity chamber for two hours at 37° C. In the later part of the project the smears were allowed to dry in air for up to six hours before being covered with the incubation medium. After incubation the preparations were washed in distilled water and mounted in Farrants's medium.

Media for glucose-6-phosphate dehydrogenase.—Triphosphopyridine nucleotide (T.P.N., N.A.D.P.), 0.05 g.; glucose-6-phosphate sodium salt (Boehringer), 0.15 g.; dissolved in 0.05 M phosphate buffer at pH 7.8, containing 0.1% neotetrazolium.

Media for 6-phosphogluconate dehydrogenase.—T.P.N., 0.05 g.; 6-phosphogluconate trisodium salt (Wessex), 0.05 g.; 0.1% solution neotetrazolium in 0.05 M phosphate buffer, pH 7.8, 10 ml.

As controls, duplicate smears were incubated in media which lacked either the coenzyme (T.P.N.) or the substrate (glucose-6-phosphate or 6-phosphogluconate), depending on the dehydrogenase which was being tested.

RESULTS

Glucose-6-phosphate dehydrogenase activity was demonstrated in the smears both from normal patients and from those with malignant disease. In the former, this activity was very low and was confined mainly to leucocytes and to what appeared to be groups of endocervical cells. This activity was represented by only very small and widely scattered crystals of formazan (Fig. 1).

In those smears obtained from patients with carcinoma of the cervix the enzyme activity was intense. There were numerous groups of cells with heavy deposits of formazan crystals. The numbers, the distribution, and occasionally the shape of these cells indicated that they may have been the same cells which on conventional staining would have been described as "malignant cells" (Figs. 2 and 3). Furthermore, in the smears from the malignant cases some activity was demonstrated even in cells which morphologically appeared to be normal squames. This occurred far more often and with greater intensity than in the smears from normal patients. In none of the smears from the normal cases were any cells found with an intensity of reaction comparable to that of the so-called "malignant cells."

When 6-phosphogluconate dehydrogenase activity was studied, the findings were similar to those with glucose-6-phosphate dehydrogenase, but the overall activity was rather lower.

In the initial stages of this study the smears were immediately flooded with the incubation medium for fear that any drying might affect dehydrogenase activity. To avoid any possibility of drying, the smears had to be processed in the clinic or ward where they were obtained, and this made the procedure somewhat cumbersome. In an attempt to simplify the technique, the smears from some of the later cases were allowed to dry in air for up to six hours, after which they were processed under more normal laboratory conditions. We found that the drying effect did not diminish the dehydrogenase activity. The later smears were of better quality than the earlier wet preparations because more cells adhered to the slide.

DISCUSSION

These studies have shown that it is possible to demonstrate respiratory enzyme activity on scrape smears from the uterine cervix, thus combining the techniques and advantages of exfoliative cytology with biochemistry. By so doing it may be

FIG. 1

FIG. 2

FIG. 3

FIG. 1.—Cervical scrape smear from healthy cervix stained for glucose-6-phosphate dehydrogenase activity. Note that reaction is weak and virtually limited to leucocytes. ($\times 755$.) FIG. 2.—Cervical scrape smear from carcinoma of cervix stained for glucose-6-phosphate dehydrogenase activity. Large "tadpole" cell showing heavy deposits of formazan crystals indicating intense activity. ($\times 755$.) FIG. 3.—Cervical scrape smear from carcinoma of cervix stained for glucose-6-phosphate dehydrogenase activity. ($\times 755$.)

possible to make exfoliative cytology less subjective and more decisive, and consequently easier to apply. The evidence of a characteristically high activity of enzymes of the hexose monophosphate shunt in smears from cases of known carcinoma of the cervix suggests the possibility of a "histochemical stain" which might assist the interpretation of smears.

SUMMARY

It is possible to demonstrate respiratory enzyme activity on scrape smears from the uterine cervix.

Because cancer cells show a more intense reaction for the dehydrogenases of the hexose-monophosphate pathway than normal cells, it may be possible to develop a histochemical stain to assist interpretation of smears.

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Medical Memoranda

Hydrohaematocolpos

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Hydrometrocolpos is a very rare condition and hydrohaematocolpos is rarer still. We therefore thought it worth while to place this case on record.

CASE REPORT

A full-term 5-days-old female child was referred to us for a lump in the lower abdomen, unassociated with any significant symptoms, except distension of the abdomen and occasional episodes of vomiting.

The child was examined on the sixth day after birth. It was observed that her general condition was satisfactory and that she had polydactyly of both hands on the ulnar side. The lower abdomen was distended. Palpation revealed a mid-line, intra-abdominal, non-tender cystic lump arising from the pelvis; it measured about 10 cm. by 7 cm. and had an uneven surface and well-defined borders. The upper pole was just above the umbilicus and was mobile from side to side. Rectal examination revealed a cystic mass anteriorly. No other abnormality was detected in the abdomen or in other systems. The results of investigations showed: Hb 16.2 g./100 ml.; urine, nothing abnormal; plain radiograph of the abdomen, soft-tissue shadow in the pelvis.

Since the symptoms of subacute obstruction were persistent and progressive, a laparotomy was performed on the eleventh day after birth. A large cystic swelling was found arising from the pelvis and pushing the bladder upwards and anteriorly. It was, in fact, the grossly distended uterus and upper part of the vagina. Tubes, ovaries, and other organs were found to be normal. As the condition of the child was not satisfactory, the cyst was decompressed by aspiration. About 200 ml. of blood-stained fluid was removed, the puncture site inverted with chromic catgut, and the abdomen closed in layers. Routine post-operative care was given. The child developed bronchopneumonia and wound infection, to which she succumbed on the twelfth post-operative day. Permission for necropsy was not obtained.

COMMENT

Hydrometrocolpos is a condition, always occurring before the menarche, in which the uterus and vagina are distended with retained fluid. In hydrohaematocolpos the fluid is blood-stained. In a series of 62 cases of hydrometrocolpos in different

age groups (Spencer and Levy, 1962) 48 were seen in infants below 3 months of age. Of the 48 cases only two were classified as hydrohaematocolpos. A more descriptive name for the condition described here would be hydrohaematometocolpos.

Hydrohaematocolpos is always associated with two aetiological factors—an obstruction to the vaginal canal and a hormonal factor. The condition can occur in very early infancy when there is a high level of maternal oestrogens or in early puberty when the patient begins to elaborate her own hormones. Of the causes of obstruction found in the literature, the order of frequency is imperforate hymen in 42, atresia of the vagina in 13, and transverse septum in seven cases (*Year Book of Pediatrics*, 1963-4). The cause of obstruction in the case studied here was partial atresia of the vagina, the extent of which could not be clinically assessed.

The clinical manifestations with which the patient may present are usually a lower abdominal lump or, less frequently, intestinal, urinary, or venous obstruction—either singly or in varying combinations. Cases may be asymptomatic and be discovered accidentally on routine examination by the presence of a bulging vaginal membrane. Except that the retained fluid is blood-stained, there is no difference in the symptomatology of hydrohaematocolpos and hydrometrocolpos.

Treatment should be directed towards permanent relief of obstruction to the vaginal tract. Wherever patency can be established by surgery it is recommended that a small rubber drainage tube should be left in place for a few days, but not for too long because of the risk of infection. Resection of the uterus should be avoided, because it leaves behind a permanent problem.

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