A NEW ENZYME TEST FOR GYNAECOLOGICAL CANCER

6-PHOSPHOGLUCONATE DEHYDROGENASE ACTIVITY IN VAGINAL FLUID

BY

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The ease of collection of preparations of exfoliated cells from the female genital tract has facilitated the recent widespread interest in cytodiagnostic tests for cancer, particularly of the cervix uteri. If cervical cells are readily available for cytological preparations they are also accessible to biochemical study. Such a biochemical study of exfoliated malignant and premalignant cells holds promise not only of an alternative screening technique but also of fundamental discoveries in the field of the metabolism of the malignant cell, as was emphasized by Nixon (1959).

Most of the work done on the biochemical changes in vaginal fluid in malignant conditions of the cervix has been confined to the increased activity of β -glucuronidase. Following reports of increased β -glucuronidase activity in malignant tissue, Odell and Burt (1950) confirmed these findings in vaginal fluid of patients with carcinoma of the cervix and suggested the possibility of a diagnostic test. At the same time Fishman, Kasdar, and Homburger (1950) published the first of a series of articles on the β -glucuronidase levels in the vaginal fluid of patients with benign conditions and with pre-invasive and invasive carcinoma. Both types of malignant lesions had raised β -glucuronidase levels. Lawson (1959) published similar results of β -glucuronidase assay, and in the following year estimations of α -mannosidase activity (Lawson, 1960). He suggested the additional value of enzyme assay in estimating prognosis. Hatzimichael (1962) has recently reported raised β -glucuronidase activity in cervical secretion from cervical-cancer patients.

Despite a modified technique, giving a higher yield of the β -glucuronidase from the cells where it is almost exclusively found (Nixon, Bonham, and Gibbs, 1962), we have noted an overlap in range of enzyme activity between patients with cancer and those with benign conditions. Estimations of β -glucuronidase therefore did not appear to provide the required specificity for a cancer screening test, and we have recently turned our attention to alternative enzyme assays. Following the report by Chayen (1961) of a high level of 6-phosphogluconate dehydrogenase (6-PG dehydrogenase) in regenerating liver cells, hyperplastic bronchial epithelium, and squamous bronchial epithelium, we turned our attention to the estimation of this enzyme in vaginal fluid. The early results appear most encouraging and justify an interim communication. The enzyme 6-PG dehydrogenase is concerned in carbohydrate metabolism by the pentose pathway. Increased pentose-pathway metabolism by malignant tissues has led Sahasrabudhe (1958) to suggest cancer chemotherapy with analogues of 6-phosphogluconic acid. Our estimation of 6-PG dehydrogenase has utilized the TPN-

TPNH change by a method modified from that of Glock and McLean (1953).

Technique

Prior to routine gynaecological examination, or prior to cleansing of the vagina in operation cases, a specimen of vaginal fluid is collected blind from the posterior fornix by oral suction into a capillary tube of internal bore 1.5 or 3 mm. The narrower the tube the more effective it is in older patients where the volume of fluid is small. Oral suction has proved more controllable than bulb suction, confirming the findings of Lawson (1959). The specimen is transferred to pre-weighed sample tubes. Where the sample is small the addition of a small volume of glass-distilled water to the sample tube permits rinsing out of the collecting capillary. The specimens are then freeze-dried for three hours in an Edwards 5 PS centrifugal unit, after which they are weighed. Glass-distilled water is then added to give a suspension containing approximately 1 mg./0.1 ml. The suspensions are then centrifuged at 20,000 g. for 45 minutes.

The assay system comprises: 0.1 ml. of supernatant from centrifugation; 0.5 ml. magnesium chloride solution, 0.1 M; 0.5 ml. glycylglycine buffer pH 9.0, 0.25 M; 0.2 ml. TPN solution, 2 mg./ml.; 0.2 ml. trisodium 6-phosphogluconate, 0.02 M. The total test volume is thus 1.5 ml. and the control solution is as above but contains water instead of TPN solution.

As soon as the 6-phosphogluconate has been added the optical density of the test solution is compared with the control at 340 m μ , using 1 cm. light path at 20° C. in a Unicam S.P. 500 spectrophotometer. The change in optical density (ΔD) is read after five minutes.

Calculation

A unit of activity is defined as the amount of enzyme which reduces 0.01 u.ml. TPN/min. at 20° C. based on the change in optical density over the first five minutes.

It can be shown that, using a test volume of 1.5 ml. at 20° C.,

1 unit 6-PG dehydrogenase = ΔD of 0.418 in five minutes, therefore units 6-PG dehydrogenase/g. dry weight sample

$$= \frac{\Delta D \times 1,000}{5 \times 0.418 \times S}$$

where S is sample weight in mg. corresponding to test volume.

$$\text{Therefore units/g.} = \frac{D \times 4,784.7}{S}$$

The results are expressed as units per gramme dry weight of sample.

Results

Initially the test has been applied to 139 patients, of whom 90 had no evidence of malignancy, and 46 who had histologically proved carcinoma or other gynaecological neoplasms.

Repeated readings on many of the malignant patients have been consistent but are not here reported.

Units/g. 6-PGD Activity	No. of Patients		
	No Malignancy	Malignant	Total
0-30	90	0	90
30-100	0	0	0
Over 100	3	46	49
Total	93	46	139

The difference in activity in patients with and without malignant lesions is very highly significant and would occur by chance only one in 100,000 ($\chi^2 = 118$, $p = 0.00001$). There is no overlap between the benign and malignant groups, while between the suggested maximum for the normal patient of 30 units and the lower limit of the malignant cases at 100 units there were no readings.

Of the patients with no malignant lesion, 58 were premenopausal, 14 post-menopausal, and 21 were pregnant. Altogether there were three false-positive readings (3.2%), of which two were possibly related to trichomonas vaginitis infections, giving lower readings after treatment. This difficulty has also been reported by Kasdon *et al.* (1951) and Lawson (1959) with β -glucuronidase assay.

The 46 patients with proved gynaecological malignancy included 24 cases of squamous carcinoma, 2 cases of adenocarcinoma of the cervix, 12 cases of carcinoma of the corpus, and 8 other lesions (uterine and vaginal sarcoma, squamous carcinoma of the vagina, melanoma of the vagina, and one of squamous carcinoma of the vulva where the exudate was aspirated instead of vaginal fluid). In every case with an exfoliating lesion of the genital tract, including three cases of carcinoma-in-situ, the levels were above 100 units. The cases with carcinoma-in-situ had readings distributed at random in the malignant range.

Squamous cancer gave a range of 174–1,633 units (mean 579 units), while the adenocarcinoma range was 106–1,035 units (mean 489 units).

Biopsies of benign and malignant gynaecological tissues have also been studied after homogenization, and the results are consistent with the corresponding vaginal fluid findings.

Discussion

Despite the immense value of cytodiagnosis of early cancer of the uterus, two difficulties are inherent in the method—firstly, the experience of the technician and cytologist need to be of a high order, and, secondly, the certainty of diagnosis of corporeal cancer is not as great as that of the cervix. It is suggested that a biochemical test of vaginal-fluid-enzyme activity promises a simpler and more consistent test for screening, and in addition may provide useful evidence of the metabolic change in the cancerous and precancerous cell possibly at an ever earlier stage than histologically accepted carcinoma-in-situ. To date the 6-phosphogluconate activity promises to be a reliable enzyme test. The biochemical technique is not difficult, and it is estimated to cost no more than 5s. per test apart from capital expenditure on apparatus, as well as lending itself to possible automatic analysis. The possibilities of response to treatment and follow-up are also being studied as well as any relation to histological grading and clinical stage. The occasional case of the false positive, possibly related to trichomoniasis, will require additional study.

Summary

A new enzyme test as an aid in the diagnosis of gynaecological cancer is described using the increased activity of 6-phosphogluconate dehydrogenase in vaginal fluid.

In 93 non-malignant cases there were three false positives (3.2%).

In 46 malignant cases there were no false negatives.

The diagnosis of cancer appeared to be equally as effective in cases with corporeal adenocarcinoma and mesodermal tumours as with carcinoma of the cervix, including carcinoma-in-situ.

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CAESAREAN SECTION IN A PRIMITIVE COMMUNITY

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There appears to be a widely held belief that caesarean section in a primitive community is to be avoided if at all possible, primarily because of the risks that the scar may rupture in a future pregnancy before the patient reaches hospital. In order to test the validity of this assumption a survey has been made of all cases of previous caesarean section admitted to an up-country hospital in Uganda during January, 1959, to June, 1961, inclusive. At the same time a survey has been made of all cases of caesarean section over the same period.

Locale

The town of Mbale, which is the provincial headquarters of the Eastern Province of Uganda, has an African population of 12,000 and is expanding fairly rapidly. The hospital, however, also serves all the area for 20 to 30 miles (32 to 48 km.) around, which includes four Government maternity centres staffed by locally trained African midwives, plus a couple of similar mission centres. The maternity centres are all situated in dense agricultural areas at some distance from the main roads. Consequently there may be quite a long delay in transferring patients to hospital, and in addition these patients are often already in a poor condition when they reach the maternity centres.

The people are of various tribes, but fall roughly into two main groups: the Southern Bantu with their small pelvises, and the Nilotic or Northern tribes, who are generally of larger stature and seldom have difficulty in labour. The profusion of tribes is matched by the variety of languages, and this often leads to difficulty in obtaining a satisfactory history: certainly in cases of previous caesarean section it is only occasionally possible to find the reason why the operation was done and