Preliminary Communications

Diagnostic Test for Cervical Cancer

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Since the first report by Bonham and Gibbs (1962) on the value of vaginal fluid 6-phosphogluconate dehydrogenase (6-PG dehydrogenase) activity in the diagnosis of cancer of the uterine cervix, only two of the many papers evaluating this test have been favourably inclined to the using of this estimation for routine cancer screening (Moukhtar and Higgins, 1965; Wootton and Shepperd, 1967). In the hands of most workers the test as proposed by Bonham and Gibbs (1962) gives an unacceptably high false-positive rate and is negative in a high percentage of intraepithelial carcinomas.

Though Lawson and Watkins (1965) advised that much greater developmental work was needed before enzyme tests could be applied with confidence to the screening of mass populations for cervical cancer, the method of estimating 6-PG dehydrogenase has been virtually unaltered since it was first described. Before the estimation of enzyme activity may be carried out a sample must be collected from the fornices by suction applied to a micropipette, the material washed out, freeze dried, accurately weighed, resuspended, and spun down at high speed in a refrigerated centrifuge. All of these steps are time-consuming. They call for skilled technical manipulation and expensive apparatus, and, while the estimation of 6-PG dehydrogenase activity in the final supernatant can be automated (Cameron and Husain, 1965), this advantage is limited by the occurrence of "bottle-necks" during preparation of the specimen for analysis.

During a study of the 6-PG dehydrogenase activity in material obtained by irrigation of the vagina we observed that a high percentage of the activity in cancer subjects was associated with the extracellular vaginal fluid, and that from the standpoint of cancer diagnosis there was no advantage in using the intracellular supernatant fluid (Goldberg *et al.*, 1967). This study was based on pooled material obtained from groups of 5 to 10 subjects, and has led to a simplified 6-PG dehydrogenase test which we are applying to individual subjects. Our results to date are few, but they indicate that the simplified test approaches the accuracy of the original procedure and is more suitable for the screening of large populations. The present communication is intended to attract the attention of workers already engaged in such programmes so that a rapid evaluation of this test can be made, as we shall have access to hospital patients for a limited time.

Materials and Methods

Thirty unselected patients referred to a hospital gynaecological clinic with the complaint of vaginal discharge and/or bleeding were examined. None was found to have primary genital carcinoma, and the final diagnoses are given in Table I. Their ages ranged from 17 to 73; 18 were premenopausal and 12 postmenopausal. Twenty-five subjects awaiting treatment for cancer of the uterine cervix were also examined. Their ages ranged from 31 to 74; 10 were premenopausal and 15 postmenopausal. They were graded according to the international classification as set out in Table II.

TABLE I.—*Final Primary Diagnosis in 30 Subjects Free of Genital Cancer*

Diagnosis	No. of Cases
Erosion of cervix	4
Chronic cervicitis	4
Chronic cervicitis + cervical polyp	5
Trichomonal vaginitis	3
Senile vaginitis	3
Dysfunctional bleeding	3
Fibroids	3
Pruritus vulvae	1
Stenosis of cervical canal	1
Cancer of stomach	1
No abnormality found	2

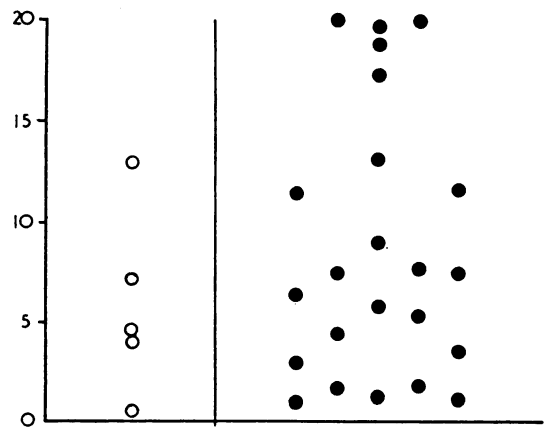
TABLE II.—*Grading of 25 Subjects with Cancer of the Cervix Uteri*

Stage	I	IIa	IIb	III	IV
No. of cases	7	3	3	7	5

With the patient in the lithotomy position, 5 ml. of sterile saline was forcefully directed towards the cervix by means of a plastic syringe to the tip of which a plastic disposable female catheter was attached. After five successive episodes of withdrawing and reinjecting the fluid, a sample was sucked back, 1 ml. being the minimum required. This was filtered through Whatman No. 42 paper, and 6-PG dehydrogenase activity of the filtrate was measured by the method of Ayre and Goldberg (1966). By following the initial rate of reduction of nicotinamide adenine dinucleotide phosphate at *p*H 7.4 and 25° C., activity was expressed as mμM substrate oxidized/min./ml. Where positive activity was recorded the protein content of the fluid was determined by the method of Lowry *et al.* (1951). Enzyme activity was divided by protein content to give the activity/mg. protein (specific gravity).

Results

With the exception of five of the non-malignant cases no 6-PG dehydrogenase activity was detected in the extracellular vaginal fluid of this group. All but 2 of the 25 cancer cases had measurable 6-PG dehydrogenase activity, the mean for the entire group being 8.34 units/mg. protein and the S.D. ± 7.87. All positive results are shown in the Chart. Clinical details of the five false-positive non-malignant cases and the two false-negative cancer cases are given in Table III. We did not have an opportunity of re-examining the false-positive cases, but



Extracellular 6-PG dehydrogenase activity of vaginal fluid from 30 subjects free from genital malignancy (open circles) and 25 with cancer of the cervix uteri (solid circles). The 25 non-malignant and two malignant subjects in whom enzyme could not be detected are not included. Results are mμM substrate oxidized/min./mg. protein at 25° C.

when the two false-negative cases were re-examined after one to two weeks activities of 7.76 and 4.16 were obtained.

TABLE III.—*Details of False-positive and False-negative Cases*

Age	Phase of Cycle	Appearance of Fluid	Primary and Secondary Diagnosis
32*	Mid	Polymorph exudate	Erosion of cervix
45*	Early	Grossly blood-stained	Fibroids. Endometrial polyp + cervical polyp + chronic cervicitis
73*	Post‡	Grossly blood-stained	Cancer of stomach + peritoneal secondaries
21*	Mid	Normal	No abnormality found
30*	Mid	Blood + inflammatory exudate	Trichomonal vaginitis + erosion + chronic cervicitis
64†	Post‡	Inflammatory exudate	Stage III well-differentiated squamous carcinoma
38†	Post‡	Normal	Stage I epidermoid carcinoma

* False-positive. † False-negative. ‡ Postmenopausal.

DISCUSSION

The test described is rapid and simple. A result may be obtained within 30 minutes of collecting the sample. The only skill required is during the estimation of enzyme activity, and this analysis can now be automated (Cameron and Husain, 1965). Technically the requirements of a screening procedure are met.

In the present series the false-positive rate was less than 20%, and this compares favourably with most published series involving conventional 6-PG dehydrogenase assay. Three of the five patients concerned gave heavily blood-stained fluid, and on these grounds a full gynaecological examination was in any case required. Four of the five were premenopausal; this contrasts with the high rate of postmenopausal false-positive cases reported by others for the conventional test (Kolstad *et al.* 1967). Though a higher rate of false-positives might be expected in this series of referred patients than in the general population, the incidence of *Trichomonas vaginalis* infection was not high, and this condition is associated with the highest rate of false-positive results (Hoffman and Merritt, 1965; Bell and Egerton, 1965). Only one of our three cases had detectable extracellular 6-PG dehydrogenase activity.

The false-negative rate in the cancer group was lower than all but three of the published series using conventional 6-PG dehydrogenase estimations (Bonham and Gibbs, 1962; Moukhtar and Higgins, 1965; Cameron and Husain, 1965). These cases had been fully diagnosed at the time the test was carried out, and we cannot extrapolate these results to population screening. More than one-quarter of the cases had stage I carcinoma, only one of which gave a false-negative result when first examined. But we were previously unable to detect extracellular 6-PG dehydrogenase in a single pooled sample from patients with intraepithelial lesions (Goldberg *et al.*, 1967).

Recently, examination of cervical mucus (Brooks and Muir, 1967) and measurement of total 6-PG dehydrogenase activity of the vaginal fluid with reference to its protein content (Sanner *et al.*, 1967) have been proposed for cancer detection, but the results obtained have not been especially promising. The extracellular 6-PG dehydrogenase activity seems to us to warrant further study. As the specific activities of the malignant and non-malignant groups overlap, there is no advantage in measuring the protein concentration, so that the test becomes virtually qualitative for the detection of 6-PG dehydrogenase activity in the extracellular fluid of the vagina. At present we can offer no explanation for the mechanism of this change in cancer subjects.

Finally, it would be unfortunate if reliance on cytological techniques were to obscure the desirability of evaluating and developing chemical methods of cancer detection. After more than two decades the false-negative rate for cytological screening still varies between 2.4 and 19.0% (Richart, 1964), the higher rates being associated with vaginal pool material such as that employed in the present study (Bredahl *et al.*, 1965).

SUMMARY

Phosphogluconate dehydrogenase activity was measured in extracellular fluid obtained from vaginal washings after filtration. Preliminary results with this simplified technique, which is suitable for large-scale screening procedures, show the test to be as reliable an index for the diagnosis of cervical cancer as previous methods employing the more difficult assay of the intracellular enzyme.

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REFERENCES

Ayre, H. A., and Goldberg, D. M. (1966). *Brit. J. Cancer*, **20**, 743.
Bell, J. L., and Egerton, M. E. (1965). *J. Obstet. Gynaec. Brit. Cwlth*, **72**, 603.
Bonham, D. G., and Gibbs, D. F. (1962). *Brit. med. J.*, **2**, 823.
Bredahl, E., Koch, F., and Stakemann, G. (1965). *Acta cytol. (Philad.)*, **9**, 189.
Brooks, P. C., and Muir, G. G. (1967). *J. Obstet. Gynaec. Brit. Cwlth*, **74**, 111.
Cameron, C. B., and Husain, O. A. N. (1965). *Brit. med. J.*, **1**, 1529.
Goldberg, D. M., Hart, D. M., and Watts, C. (1968). *Cancer (Philad.)*, **21**, 524.
Hoffman, R. L., and Merritt, J. W. (1965). *Amer. J. Obstet. Gynec.*, **92**, 650.
Kolstad, P., Bergsjø, P., Koller, O., Pihl, A., and Sanner, T. (1967). *Amer. J. Obstet. Gynec.*, **98**, 804.
Lawson, J. G., and Watkins, D. K. (1965). *J. Obstet. Gynaec. Brit. Cwlth*, **72**, 1.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951). *J. biol. Chem.*, **193**, 265.
Moukhtar, M., and Higgins, G. (1965). *J. Obstet. Gynaec. Brit. Cwlth*, **72**, 677.
Richart, R. M. (1964). *Amer J. Obstet. Gynec.*, **89**, 723.
Sanner, T., Bergsjø, P., Koller, O., Kolstad, P., and Pihl, A. (1967). Ibid., **98**, 800.
Wootton, I. D. P., and Shepperd, Y. (1967). *J. Obstet. Gynaec. Brit. Cwlth*, **74**, 270.