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Vaginal fluid enzyme patterns in benign and malignant lesions of the female genital tract

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SYNOPSIS The enzymes phosphohexose isomerase, lactate dehydrogenase, hydroxybutyrate dehydrogenase, isocitric dehydrogenase, glutamate dehydrogenase, and sorbital dehydrogenase were assayed in cases of known carcinoma of the cervix and of carcinoma *in situ*. Phosphohexose isomerase, lactate dehydrogenase, and isocitric dehydrogenase were also assayed in a number of normal women and in those with benign lesions. It is unlikely that vaginal fluid enzymology will provide a screening test for cervical carcinoma.

The success of exfoliative cytology in detecting pre-invasive carcinoma of the cervix has stimulated research into more simply applied means of detecting this condition. The work of Odell and Burt (1950), Fishman, Kasdon, and Homburger (1950), and of Bonham and Gibbs (1962), coupled with the rapid development of automated enzymological assays, suggested the possibility of using vaginal fluid enzyme levels. Most of the reports until now have not shown that any one enzyme fulfilled the requirements for either a screening or diagnostic technique. In this work the activities of phosphohexose isomerase (PHI), lactate dehydrogenase (LDH), hydroxybutyrate dehydrogenase (HBD), glutamate dehydrogenase (GLDH), isocitrate dehydrogenase (ICD), and sorbital dehydrogenase (SDH) in vaginal fluid have been studied. In the case of each enzyme there were reasons to merit its inclusion in the study.

Muir (1966) suggested that PHI might act as a screening test for cervical carcinoma, but in a survey using freeze drying in the preparation of the samples its activity did not prove reliable (Muir and Valteris, 1968). Since it was shown that freeze drying caused considerable loss of PHI activity, the enzyme has been re-evaluated. Lactate dehydrogenase activity in the vaginal fluid has been shown to give similar results to that of 6-phosphogluconate dehydrogenase (Muir, 1966). Latner (1964) and Latner, Turner, and Way (1966) showed that both in carcinoma *in situ* and invasive carcinoma there was a change in isoenzyme pattern towards the predominance of the slower moving isoenzymes. In this work the total LDH and the ratio of HBD/LDH have been studied as it was hoped that the latter might reduce the number of false positive results.

Ayre and Goldberg (1966) found raised levels of ICD in carcinomatous cervical tissue. Glutamic dehydrogenase is a link enzyme between carbohydrate and amino acid metabolism, and in certain cases of leukaemia or cancer (Waisman, Monder, and Williams, 1956) raised serum levels have been found, and Rimbach and Bonow (1961) demonstrated raised serum sorbitol dehydrogenase activity in patients with uterine carcinoma.

MATERIALS AND METHODS

Samples were obtained from local well women's clinics, the hospital Gynaecological Outpatient Department, and the Radiotherapy Department.

Material was collected using the method of Bonham and Gibbs (1962) and samples were kept at −20°C until they were assayed.

PREPARATION OF SAMPLE Nonidet P.40 was used to effect the release of enzymes from the vaginal fluid. A 10% concentration in water gave maximum enzyme release. Samples of vaginal fluid were mixed on a Rotamixer[1] until a homogeneously dispersed solution was obtained. Aliquots were pipetted into plastic centrifuge tubes, and equal aliquots of 10% nonidet added; the tubes were then mixed for a further five seconds. After 10 minutes' centrifugation at 10,000 *g* at 4°C the supernatants or suitable dilutions were assayed for enzyme activity. Potassium estimations were made on a 1 : 20 dilution of the supernatant. The potassium standard was made up in 0·25% nonidet P.40 to allow for the potassium content of the detergent. Enzyme activities were expressed

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as milli international units per milliequivalents of potassium.

PHOSPHOHEXOSE ISOMERASE (EC.5.3.1.9. D-GLUCOSE-6-PHOSPHATE KETOL ISOMERASE) The method of Horrocks, Ward, and King (1963) was used. Supernatant vaginal fluid, 0·1 ml, was taken and further diluted when the activities found were greater than 250/mU/ml of supernatant.

LACTATE DEHYDROGENASE (E.C.1.1.1.27 L-LACTATE : NAD OXIDOREDUCTASE) AND HYDROXYBUTYRATE DEHYDROGENASE (1.1.1.30 D-3-HYDROXYBUTYRATE : NAD OXIDOREDUCTASE) Automated fluorimetric assays based on the ultraviolet assays of Wróblewski and LaDue (1955) and Rosalki and Wilkinson (1960) devised by Glenn (personal communication) were used. The supernatant of the vaginal debris was diluted 1 : 20 with saline for this assay. Extremely active samples required further dilution.

ISOCITRIC DEHYDROGENASE (E.C.1.1.1.42 THREO-D-ISOCITRATE : NADP OXIDOREDUCTASE (DECARBOXYLATING)) The method of Bell and Baron (1960) was used for assaying this enzyme, using 0·2 ml vaginal fluid supernatant instead of 0·2 ml serum.

SORBITOL DEHYDROGENASE (E.C.1.1.1.14 L-IDITOL; NAD OXIDOREDUCTASE) The C. F. Boeringer test combination based on the assay of Gerlach and Schürmeyer (1960) was used. The vaginal supernatant was diluted to give a volume of 1 ml.

GLUTAMATE DEHYDROGENASE (E.C.1.4.1.2. L-GLUTAMATE: NAD OXIDOREDUCTASE (DEAMINATING) The C. F. Boehringer test combination based on the assay of Gerlach (1957) was used. The vaginal supernatant was diluted to a volume of 1 ml for the assay.

CALCULATION OF RESULTS

The results of all the enzyme activities were obtained in mU/ml. The enzyme activity per millilitre was divided by potassium concentration per millilitre to give the enzyme activity per milliequivalent of vaginal fluid potassium. Since potassium is predominantly an intracellular electrolyte it has been used to relate the enzyme activity to the cellularity of the vaginal fluid debris (Labrum, Gibbs, and Stagg, 1967).

RESULTS

PHOSPHOHEXOSE ISOMERASE Phosphohexose isomerase activity was assayed in 81 samples from normal women and women with benign gynaecological lesions, in 23 samples from patients with carcinoma of the cervix, and in two samples from patients with carcinoma *in situ* (Table I). Most samples had some enzyme activity but all samples from patients with carcinoma of the cervix and carcinoma *in situ* had activities above 100/mU/m-equiv. However, 43% of samples associated with normal and benign lesions were also above this level. The median value for PHI activity found in the vaginal fluid in patients with malignant lesions was significantly higher (620/mU/m-equiv K+) than in patients with benign lesions (76 mU/m-equiv K+).

LACTATE DEHYDROGENASE AND HYDROXYBUTYRATE DEHYDROGENASE The LDH activities were studied in 193 samples from women with no gynaecological lesions or benign lesions and samples from 23 known cases of carcinoma of the cervix and two of carcinoma *in situ* (Table II). All the cases of carcinoma had values above 100 mU/m-equiv except one of the two samples from cases with carcinoma *in situ* had activities below this level. If 100 mU/m-equiv were taken as the level at which one suspected carcinoma there would be 25% (25/98) false positive in well women and 41% (39/95) in women with gynaecological lesions, the overall false positive rate being 30%.

No useful distinction could be made betwee

TABLE I

PHOSPHOHEXOSE ISOMERASE ACTIVITY IN SPECIMENS FROM NORMAL WOMEN AND IN VARIOUS GYNAECOLOGICAL CONDITIONS

Enzyme Activity (mU/m-equiv k+)	*No. of Women with*			
	Normal Women	*Benign Gynaecological Lesions*	*Cervical Carcinoma*	*Carcinoma* in situ
Over 180	19	10	21	1
161-180	—	2	1	—
141-160	—	1	—	—
121-140	3	—	—	1
101-120	—	1	1	—
81-100	2	—	—	—
61-80	2	3	—	—
41-60	8	2	—	—
21-40	6	3	—	—
0-20	7	12	—	—
Total	47	34	23	2

TABLE II

LACTATE DEHYDROGENASE ACTIVITY IN VAGINAL FLUID OF VARIOUS GYNAECOLOGICAL CONDITIONS

Enzyme Activity (*mU/m-equiv k+*)	*Normal Women*	*No. of Women with* *Benign Gynaecological Lesions*	*Cervical Carcinoma*	*Carcinoma* in situ
500 or more	6	13	14	—
401-500	4	4	—	—
301-400	3	7	3	1
201-300	7	5	4	—
101-200	5	15	2	—
51-100	12	7	—	—
0-50	33	19	—	1
Nil	28	25	—	—
Total	98	95	23	2

TABLE III

RATIOS OF HYDROXYBUTYRATE DEHYDROGENASE ACTIVITY TO LACTATE DEHYDROGENASE ACTIVITY IN NORMAL WOMEN AND WOMEN WITH VARIOUS GYNAECOLOGICAL CONDITIONS

HBD/LDM Ratio (*mU/m-equiv K+*)	*Normal Women*	*No. of Women with* *Benign Gynaecological Lesions*	*Cervical Carcinoma*	*Carcinoma* in situ
More than 1·0	19	5	—	—
0·8-1·0	2	2	2	1
0·8-0·6	7	14	7	—
0·6-0·4	11	17	10	—
0·4-0·2	19	14	1	—
0 -0·2	6	1	1	1
Total	64	53	21	2

samples from patients with carcinomatous and benign lesions using the ratio of HBD/LDH activities per unit of potassium (Table III).

ISOCITRATE DEHYDROGENASE Fifty-five of 63 samples of vaginal fluid obtained from women with gynaecological lesions had some ICD activity. One sample from patients with carcinoma of the cervix had no ICD activity (Table IV). There was a considerable overlap of ICD activity in both benign and malignant lesions.

TABLE IV

ISOCITRATE DEHYDROGENASE ACTIVITY IN VAGINAL FLUID SPECIMENS FROM WOMEN WITH VARIOUS GYNAECOLOGICAL CONDITIONS

Enzyme Activity (*mU/m-equiv k+*)	*No. of Women with* *Benign Gynaecological Lesions*	*Cervical Carcinoma*	*Carcinoma* in situ
More than 10	3	7	—
9-10	Nil	1	1
6-8	3	3	—
4-6	9	3	—
2-4	11	2	1
0-2	25	—	—
Nil	12	1	—
Total	63	17	2

GLUTAMATE DEHYDROGENASE AND SORBITOL DEHYDROGENASE These enzymes were not present in eight of 21 and 10 of 20 carcinomatous samples from patients with carcinomatous lesions.

Gibbs (1968) found a strong NADase activity in the vaginal fluid during his assay of 6-phosphogluconate dehydrogenase. The *p*H of the assays used was lower than the optimum *p*H for NADase activity. Gibbs found that nicotinamide and tryptophane inhibited the activity of this enzyme. Nicotinamide and tryptophane were added to the reaction mixture in a similar manner. In no samples could any evidence be found of an increased activity in the presence of these factors. This suggests that competing NADase activity was not responsible for the absence of GLDH or SDH activity in the vaginal fluid of patients with carcinoma of the cervix.

Table V gives the enzyme activities found in 23 cases of invasive carcinoma and two cases of carcinoma *in situ*.

DISCUSSION

Phosphohexose isomerase and lactate dehydrogenase gave similar results with invasive carcinoma. The false positive rate was higher for phosphohexose isomerase (43%) than for LDH (30%).

It was disappointing that the HBD/LDH ratio did not provide any definite demarcation between samples from patients with malignant and benign lesions despite the fact very high ratios were rare in

TABLE V

ENZYME ACTIVITY IN VAGINAL FLUID FROM PATIENTS WITH CERVICAL CARCINOMA

Sample No.	Enzyme Activity (mU/m-equiv K+) PHI	LDH	HBD	ICD	GLDH	SDH	Type of Carcinoma
1	160	398	260	5·0	12·7	Nil	Cervix
2	560	610	260	8·0	1·29	Nil	Cervix
3	1,100	390	390	9·0	Nil	0·16	*In situ*
4	324	680	600	17·0	56·6	Nil	Cervix
5	120	Nil	Nil	2·0	Nil	2·8	*In situ*
6	2,700	1.100	600	—	—	—	Cervix
7	750	1,200	610	—	—	—	Cervix
8	2,830	1,730	785	—	—	—	Vagina
9	620	135	95	11·0	—	—	Cervix
10	3,100	810	400	14·5	0·4	20	Cervix
11	2,500	1,200	550	11·0	7·4	0·73	Cervix
12	3,166	270	200	14·0	3·9	0·44	Cervix
13	795	360	194	—	Nil	Nil	Cervix
14	1,450	365	202	9·8	0·75	1·2	Cervix
15	248	500	238	—	Nil	Nil	Cervix
16	3,320	500	370	7·8	Nil	Nil	Cervix
17	1,660	560	406	17·0	Nil	Nil	Cervix
18	11,700	900	446	10·6	20·2	Nil	Cervix
19	276	290	240	3·6	Nil	0·37	Cervix
20	855	250	38	Nil	Nil	1·95	Cervix
21	116	182	145	3·6	Nil	Nil	Cervix
22	292	992	765	5·0	14·3	1·6	Cervix
23	4,000	1,310	327	5·3	3·3	Nil	Cervix
24	2,400	230	57	—	10·8	0·25	Cervix
25	1,150	615	274	6·2	0·072	—	Cervix

carcinoma. Most of the ratios found in samples from patients with malignant lesions were in a similar range to those found in patients with benign lesions. As HBD is a measure of the fast moving isoenzyme components one could expect the HBD/LDH ratio to be small in cases of carcinoma. As this was not the case, the present results would suggest that most of the activity of the enzymes found in vaginal fluid is derived from an inflammatory response of the tissues to the carcinoma. This has previously been suggested by Muir and Canti (1966) and by Churchouse, Carter, and Bonham (1967). Since carcinoma *in situ* exfoliates fewer cells (Wied, Legoretta, Mohr, and Rauzy, 1962) and provokes a less extensive reaction in the cervical tissue than invasive carcinoma, these lesions are less frequently associated with raised levels of vaginal fluid enzymes.

Isocitrate dehydrogenase, sorbitol dehydrogenase, and glutamate dehydrogenase provided no possibility as a means of detecting cervical carcinoma.

None of the enzymes offered a suitable means of screening for carcinoma *in situ*. When freeze drying was avoided PHI detected all invasive and carcinoma *in situ* in this series, but the high false positive rate would preclude its use.

The original work of Fishman *et al* (1950) and Odell and Burt (1950) on the beta glucuronidase activity in vaginal fluid and the subsequent studies stimulated much interest in this field. Over the past years a number of enzymes have been studied. These have included 6-phosphogluconate dehydrogenase, glucose-6-phosphate dehydrogenase, aldolase, glutamic oxaloacetic transaminase, and alphamonosidase. All these enzymes have shown high false positive rates, frequent false negative results in carcinoma *in situ*, and occasional false negative results in invasive carcinoma.

It seems unlikely that much progress can be made in this field until the nature of carcinoma *in situ* is better understood. When the relationship between enzyme-positive carcinoma *in situ* and invasive carcinoma is understood, vaginal fluid enzymology may be able to offer an automated screening technique. It seems unlikely that vaginal fluid enzymology will ever provide a specific test for carcinoma of the cervix.

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