Papers and Originals

Cervical Screening for Carcinoma: A Comparison of Cytological and Enzyme (6-phosphogluconate dehydrogenase) Methods and Clinical Findings

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The value of cervical cytology in diagnosing early invasive carcinoma and preinvasive carcinoma-in-situ lesions of the uterine cervix is well established, and its practical application as a screening test has been demonstrated among patients attending hospital (Yule and Cameron, 1961), local authority (Jones and Metcalfe Brown, 1965), and general practice clinics (Macgregor and Baird, 1963). Long-term screening programmes among wider communities (Boyes *et al.*, 1962; Bryans *et al.*, 1964) have been associated with reported falls in the incidence of invasive cervical carcinoma in the screened population, and, while follow-up data from these long-term programmes remain limited, their accumulating experience may shortly allow a firmer assessment of whether they can achieve their ultimate aim, a major reduction in mortality. To date no screening programme has been shown to have this effect.

In this country professional opinion and public demand have combined to stimulate a recent increase in cytological services; a nation-wide service has been advocated, but a shortage of cytologists and technicians has made progress toward this end slow (*British Medical Journal*, 1965). In an assessment of the practical implications of screening all women over 20 years, with repeat examinations every five years, it has been estimated (Wilson, 1965) that, for a population unit of 250,000 (roughly the size served by a group laboratory), a service on this scale would mean screening one-fifth of the 83,000 women over 20 each year—that is, about 17,000 a year. Expanded to the country as a whole such estimates indicate a screening load likely to overwhelm available cytological services, and this gap between demand and available resources for cervical screening has been a factor adding impetus to the search for possible alternatives to cytology.

One development in recent years has been the introduction of a biochemical test that promised to be very much easier to apply and much cheaper to carry out on a mass scale. Chayen *et al.* (1962) showed that 6-phosphogluconate dehydrogenase was extremely active in several types of malignant tumour and that the presence of this enzyme could be recognized histochemically. Bonham and Gibbs (1962) looked for the presence of the enzyme in vaginal secretions and found that it was usually present in large amounts with uterine and vaginal cancer and also with cervical carcinoma-in-situ, and that it was absent (or present only in small quantities) in normal women. These results suggested the possibility of a quantitative enzyme test based on the amount of 6-phosphogluconate dehydrogenase in vaginal fluid, which might offer a useful alternative to cytological methods of screening. The results were, however, derived from a small and selected group of hospital patients and clearly required validation in a wider survey covering a more representative population. The present paper reports a screening study carried out among both hospital and general practice patients, making direct comparison between the enzyme test and standard cytological methods and allowing some further comparison with clinical findings.

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Methods

The study was carried out among women attending (*a*) the outpatient department at University College Hospital Obstetric Hospital and (*b*) cervical screening clinics held in the surgeries of four local practices. Married hospital patients aged 20–65 became eligible for inclusion on making a first attendance at one or other of the hospital's antenatal, gynaecology, or cancer-detection clinics. Non-hospital patients entered the survey on acceptance of appointments for cervical screening offered to all married women aged 20–65 on the practice lists. At both hospital and practice clinics specimens for 6-phosphogluconate dehydrogenase enzyme estimations and cytological examination were obtained from each patient during a single clinic attendance. Cytological examinations and enzyme estimations were carried out at University College Hospital.

The cytology grading used was devised specifically for the survey and did not correspond exactly with that of Papanicolaou. It was as follows:

Grade I: The smear shows no abnormality.
Grade II: The smear shows an abnormality (usually inflammatory) but nothing suggestive of malignancy.
Grade III: The smear shows cells with nuclear changes considered insufficient to indicate malignancy but requiring repetition of the examination.
Grade IV: All smears thought to show carcinoma-in-situ *or* invasive carcinoma.

Enzyme specimens were brought to the laboratory after each clinic and freeze-dried immediately. The dried specimens were weighed, resuspended in 3 ml. of distilled water, and centrifuged at 100,000 *g* for 30 minutes at 0° C. in a Christ Omikron centrifuge. The resulting supernatant was assayed for 6-phosphogluconate dehydrogenase activity either by the method of Bonham and Gibbs (1962) or, when large numbers of specimens were presented, with an autoanalyser (Cameron and Weg, 1964). Separate experiments showed the two methods to give closely similar results. Care was taken to keep specimens cold

during preparatory procedures, and all glassware used was free from heavy metal contamination.

Practice Populations

All four practices were sited within a few miles of University College Hospital, and each already had close links with the hospital. Three (Practices A, B, C) were run as group partnerships and the fourth (Practice D) was a single-handed practice with one assistant. Practices B and C belonged to the same partnership, but, having separate organizations from different surgeries, were treated as individual units in the present survey.

In each practice available records were used to prepare a nominal roll of women eligible for the study. A standard letter to these patients, over the signature of one or more of their own doctors, let them know that tests for cancer of the cervix would shortly be offered at special clinics outside normal surgery hours. The letter included a non-technical explanation of the nature and purpose of cervical screening and stressed the value of early detection and treatment of "conditions of the cervix, or neck of the womb, which might lead to cancer." Patients were encouraged to take advantage of the clinics, and in a second letter were given individual appointments offering the choice of an afternoon or evening attendance at the practice surgery. Anyone finding an initial appointment inconvenient was invited to arrange a more suitable attendance for a later clinic, and in each practice weekly clinics continued until all patients wishing to attend had done so.

All clinics at practice surgeries were undertaken by one of us (A. H. L.) assisted by a hospital nurse, while preparation of practice lists, maintenance of appointments system and records, and, where necessary, reception cover for the practice clinics were organized centrally from the Statistical Research Unit.

Response to Survey in Practices

Preliminary letters were addressed to 6,093 married women enumerated from the central records of the four practices. The number of letters returned by the post office as "gone away, address unknown" suggested that the comprehensive extraction of names from all available records had included former patients for whom old, perhaps incomplete, records remained in the central files. Others were discovered to have died or changed their doctor. The extent of these losses was further assessed at the end of the survey, when social workers visited a 30% random sample of addresses of patients making no response to either letter—that is, those who neither accepted nor actively refused the appointments offered. The information obtained from these sources—returned letters and sample visiting of non-responders—was then used to estimate total losses to the survey, standardized by age and practice, due to the patients having moved, died, or changed their doctor. Exclusion of this group left a net survey population providing a firmer base for the measurement of acceptance rates. Details of the response among practice patients are presented in Table I.

TABLE I.—*Response to Survey Among Practice Patients (All Practices Combined)*

Age (Years)	Letters Sent to Patients	Patients Estimated to have Moved, Died, or Changed Doctor		Net Survey Population	Patients Seen at Clinic	Acceptance Rate (%)
		No.	%			
20–24	286	108	37·8	178	81	45·5
25–29	795	379	47·7	416	235	56·4
30–34	762	313	41·0	449	253	56·3
35–39	754	271	35·9	483	285	59·0
40–44	721	214	30·0	507	284	56·0
45–49	618	160	25·9	458	239	52·2
50–54	644	158	24·5	486	246	50·6
55–59	537	114	21·2	423	168	39·7
60+	629	109	17·3	520	132	25·4
N.K.	347	292	84·1	55	1	(–)
All ages	6,093	2,118	34·8	3,975	1,924	48·4

An estimated 2,118 persons, or 35% of those to whom letters were sent, removed themselves from the study population by moving or changing their doctor or had died. Exclusions stemmed mainly from changes of address and were most common among younger patients, with a relative frequency declining steadily from 48% at 25–29 years to less than 20% at 60 years and over. Removal of this group, to whom letters were sent but presumably never received, left a net practice population of 3,975 women, of whom 1,924 (48%) attended one or other of the four clinics. It has to be recognized that this corrected rate may overestimate response in that some letters may have been sent on to new addresses. It is believed, however, that this was probably a rare occurrence, and that the true response rate lies much closer to the estimated 48% than the uncorrected figure (32%) relating response to number of letters sent. Corrected age-specific acceptance rates displayed a minor peak (59%) at 35–39 years, but maintained a fairly stable level of over 50% between 25 and 54 years before declining steeply in the two oldest age groups. Individual practices showed the same general age pattern but differed in the overall response to the survey by their patients. Acceptance rates at all ages were highest (62%) in Practice C and lowest (42%) in Practice B. Practices A and D occupied intermediate positions with rates of 48 and 51% respectively.

Visits paid to the sample of non-responders also allowed some assessment of reasons for not accepting appointments (Table II). Almost a third of all patients, or 24–37% in individual practices, volunteered only indefinite reasons for non-attendance—for example, "must have forgotten appointment," "didn't think test really necessary," "too old," "didn't receive appointments," the last-mentioned from patients found at addresses to which letters had been sent. A second group, 10% of the combined practice population, gave no reason and have been classified as straight refusals. While a common characteristic of both these groups was clearly their lack of interest in the screening facilities offered, their answers did not provide any firm indication of possible underlying reasons for this lack of interest. Some may have shared the doubts of those giving more specific reasons for non-attendance—for example, the fear of cancer or dislike of examination expressed by 13 and 10% respectively of non-acceptors.[1] Fear of cancer was given as a reason for non-attendance by 17% of patients in Practice B, which had the lowest acceptance rate, compared with only 3% in Practice C, the practice with the highest acceptance rates. Dislike of examination was also more frequently encountered in Practice B (10%) than in Practice C (1%). Conversely, the proportions of patients giving histories of previous cyto-

TABLE II.—*Distribution (%) of Reasons for Non-attendance Among Practice Patients*

Stated Reasons for Non-attendance	Proportion of Non-responding Patients (%)				
	Practice				All Practices
	A	B	C	D	
Not eligible (single, wrong age)	3	7	9	5	5
Pregnant	4	5	6	2	4
Already had test	10	6	12	13	9
Previous hysterectomy	5	3	11	13	6
Fear of cancer	13	17	3	10	13
Dislike of examination	11	10	1	15	10
Inconvenient: illness, domestic crisis	16	9	12	9	13
Indefinite	24	37	35	24	30
Straight refusal, i.e., no reason	14	6	11	9	10
Total	100	100	100	100	100
No. of patients	888	684	236	243	2,051

[1] Though no specific inquiry was made from patients *attending* the clinics, general impressions suggested that some who attended had similar doubts; for example, the comment volunteered by one doctor "that . . . anxieties displayed by (his) patients . . . were far greater than those at a normal surgery."

logical examination and of previous hysterectomy were higher, at 12 and 11%, in Practice C than in Practice B, where comparable proportions were 6 and 3% respectively.

Results of Screening in Practice Clinics

Thirteen cases of carcinoma-in-situ among 1,924 patients attending the practice clinics gave an overall yield of 6.8 cases per 1,000 screened. Two additional cases were positive on cytological screening but negative on subsequent biopsy, and the survey also picked up a previously undetected invasive carcinoma. Prevalence of carcinoma-in-situ (Table III) was low at 3 per 1,000 among patients in their twenties, rose to a peak of 9 per 1,000 among women aged 30–39, and declined to a level of 7–8 per 1,000 in the age group of 40–59 years. No positive cases were found among the 132 women over 60 years. Among individual practices the prevalence of carcinoma-in-situ was highest in Practice B with a rate at all ages of 10.2 per 1,000, and lowest in Practice C, where the comparable rate was 2.6 per 1,000. Practices A and D had intermediate rates of 6.2 and 8.0 per 1,000 respectively.

TABLE III.—*Results of Screening Among Practice Patients by Age*

Age (Years)	Patients Screened	Carcinoma-in-situ	
		No.	Rate per 1,000
20–29	316	1	3·2
30–39	538	5	9·3
40–49	523	4	7·6
50–59	414	3	7·2
60+	132	0	(—)
Total	1,924*	13	6·8

* Including one patient of unknown age.

An estimate of the social class distribution of three of the four practice populations was derived from information on husbands' occupations obtained from a random sample (30%) of women attending the clinics. While these distributions, based on persons *accepting* appointment, cannot be taken as completely representative of all registered married women patients, they provide a useful measure for broad comparisons between practices. The data were clearly consistent with an association between social class and both acceptance and prevalence rates (Table IV). The practice (C) with the highest proportion of persons in social classes I and II had the most favourable response rate and the lowest prevalence rate, while the greatest yield of positive cases came from Practice B, which had a low acceptance rate and a social class distribution more heavily weighted with classes IV and V. The other practice (A) for which data on social class were available occupied an intermediate position for all three indices.

TABLE IV.—*Prevalence of Carcinoma-in-situ Related to Acceptance Rates, and Social Class Distributions, by Practice*

	Practice			
	A	B	C	D
Prevalence rate per 1,000 ..	6·2	10·2	2·6	8·0
Acceptance ,, ,, ,, ..	47·5	41·8	61·6	50·7
Social class distribution (%)				
I and II	34	29	75	N.K.
III	48	46	22	,,
IV and V	18	25	3	,,

Other Findings among Practice Patients

Routine recording of patients' histories and findings on vaginal examination allowed some estimate of prevalence by age for each of the conditions listed in Table V. For example, the patients' histories suggested that more than half (547 per 1,000) of the women aged 20–29 had a vaginal discharge, and that the prevalence of this symptom declined with age to a rate of 220 per 1,000 at 60 and over. The proportion of discharges described as irritating also varied with age but in an inverse manner, being lower in the third, fourth, and fifth decades (29, 18, and 29% respectively) than at older ages, where the proportion of discharges so described rose to 44% at 44–49 and to 65% among persons over 60. Cervical erosion was another common finding among younger women, with a prevalence of 424 per 1,000 at 20–29 years. Among older patients prevalence declined, gradually at first and then more rapidly, to rates of 85 and 38 per 1,000 in the sixth and seventh decades respectively. Cervical polyps, on the other hand, were rare in young women, being noted in only one patient under 30, and their frequency increased steadily with age to reach a prevalence of 98 per 1,000 at 60 years and over. With respect to *Trichomonas vaginalis* infections, routine cytological examinations uncovered a higher prevalence than was evident from clinical screening. As measured by cytology findings, prevalence of trichomonas infection increased with age to a peak of 73 per 1,000 at ages 40–49 and then fell in the two older age groups.

TABLE V.—*Other Findings Among Practice Patients by Age*

Findings	Prevalence Rates* per 1,000 at Ages:				
	20–29	30–39	40–49	50–59	60+
From patient's history:					
Vaginal discharge	547	511	436	295	220
Irritating vaginal discharge (included above)	158	93	128	130	144
On clinical examination:					
Cervical erosion	424	377	249	85	38
,, polyp	(3)	26	40	70	98
Chronic cervicitis	(13)	33	52	(5)	(15)
Vulvitis	28	24	29	51	76
Atrophic vaginitis	—	—	10	164	227
Other vaginitis	47	24	34	60	45
Trichomonal vaginitis ..	19	13	11	(10)	(8)
On cytological examination:					
Trichomonas vaginalis	25	45	73	58	(15)

* Rates in parentheses based on fewer than 5 cases.

Results of Screening among Hospital Patients

Fifteen cases of carcinoma-in-situ were discovered among 2,461 hospital outpatients, an overall yield of 6.1 per 1,000. In addition, a further suspected in-situ carcinoma, positive on initial screening but reverting to negative before biopsy, and eight clinical carcinomas of cervix or adenocarcinomas of corpus uteri were observed. Four of the 15 carcinomas-in-situ occurred among antenatal patients (3.4 per 1,000), nine among gynaecological outpatients (8.8 per 1,000), and the remaining two among women attending a cancer detection clinic (7.8 per 1,000). Age-specific prevalence (Table VI) was highest at 11.8 per thousand among patients aged 40–49, followed by a rate of 8.0 per thousand at 30–39 years. Prevalence was lower among younger women, with a rate of 4.0 per 1,000 at 20–29 years, and no in-situ carcinomas were found among the 266 women aged more than 50.

TABLE VI.—*Results of Screening Among Hospital Patients by Age*

Age (Years)	Patients Screened	Carcinoma-in-situ	
		No.	Rate per 1,000
Under 20	22	0	(—)
20–29	992	4	4·0
30–39	754	6	8·0
40–49	425	5	11·8
50–59	200	0	(—)
60+	66	0	(—)
N.K.	2	0	(—)
Total ..	2,461	15	6·1

Comparison between Enzyme Estimation and Cytological Examination

The results of paired enzyme and cytology screenings allowed direct comparison between the two methods and, supplemented

by further detail on positive cases, permitted some assessment of their relative frequencies of "false" negatives and positives. Technical failures in collection of specimens or in the laboratory were responsible for the absence of enzyme results for 457 patients (10.4% of the survey population) and of cytology results for a further 297 women (6.8%). Paired results were available for the remaining 3,631 women (82.8%).

Distribution of Enzyme and Cytology Results (Table VII)

Of 3,631 women with paired tests, 2,097 (58%) had a completely negative enzyme result—that is, nil units. A further 875 (24%) were in the enzyme range of 1–74 units. Of remaining tests, 85 (2%) gave results between 75 and 99 units, 431 (12%) between 100 and 499 units, and 143 (4%) 500 or more units. Allowing for the changing class interval adopted in Table VII, there was no clear evidence of any bimodality in this distribution.

TABLE VII.—*Distribution of Paired Enzyme and Cytology Findings (Combined Practice and Hospital Data)*

Enzyme Result (Units)	No. of Patients with given Cytology Grading				Unsatisfactory	Total
	I	II	III	IV		
Nil	1,376	626	39	8	48	2,097
1–24	273	143	8	1	10	435
25–49	154	100	9	3	15	281
50–74	85	63	5	2	4	159
75–99	49	35	0	0	1	85
100–199	94	89	3	5	4	195
200–299	57	52	2	0	0	111
300–399	26	35	5	2	6	74
400–499	19	30	0	0	2	51
500+	48	85	4	1	5	143
Total	2,181	1,258	75	22	95	3,631

Of cervical smears 95 (3%) were classified as unsatisfactory, 2,181 (60%) were graded I, 1,258 (35%) graded II, 75 (2%) graded III, and 22 (0.6%) graded IV. In addition to these 22 grade IV cytology cases, a further 18 patients yielded positive cytological or histological findings during the survey. No enzyme results were available for two of the latter, and the remaining 16 had initial smears graded II (1 patient) or III (15 patients). All cases with positive findings are listed in Tables VIII and IX, which detail enzyme and cytology results associated with diagnoses of carcinoma-in-situ and invasive cancer respectively. Diagnoses in each category have been histologically confirmed for all except three suspected carcinomas-in-situ.

Case 6.—Woman aged 32. Large florid erosion and suspicious cervical smear (February 1965). Negative punch biopsy followed by two positive smears. Most recent smear (November 1965) remained suspicious.

Case 12.—Woman aged 34. Positive smear (June 1965) followed by negative biopsy. Most recent smear (January 1966) remained suspicious.

Case 17.—Woman aged 30. Positive smear reverting to negative on follow-up. Biopsy not performed.

"False" Negatives

Carcinoma-in-situ Data.—No enzyme result was available for Case 23, and histological confirmation of the diagnosis was lacking for Cases 6, 12, and 17. There remained 27 cases of carcinoma-in-situ in which enzyme results were available. Earlier practice (Bonham and Gibbs, 1962) was followed, and using the arbitrary level of 100 units as a dividing line we found among these 27 cases 19 (70%) with a negative result (<100 units) on first testing. Among 15 cases with at least two enzyme estimations 10 (67%) were consistently negative by this standard. One previously known patient with carcinoma-in-situ (Case 20), who entered the survey on becoming pregnant and making a first attendance at the antenatal clinic, had two completely negative enzyme tests. Before entry to the survey, however, she had positive enzyme results ranging from 128 to 708 units. The single false-negative cytology (Case 19) occurred in a patient presenting with cervical polyps. Her cytology report (grade II) noted the presence of trichomonas infestation, and a concurrent estimation of enzyme activity was 409 units. Biopsy of the cervix was carried out during polypectomy, and

TABLE VIII.—*Enzyme and Cytology Results Relating to Carcinoma-in-Situ*

No.	Age	Date	Enzyme Result (Units)	Cytology Grade	Histological Confirmation?
		Practice Patients			
1	33	19/11/64	39	IV	Yes
		3/12/64	42	IV	
2	30	19/1/65	49	III	
		21/2/66	—	IV	"
		16/3/66	—	IV	
3	43	25/1/65	304	—	
		22/2/65	200	IV	"
4	48	3/2/65	215	III	
		24/2/65	168	III	
		1/6/65	—	III	
5	59	4/2/65	—	IV	
		25/2/65	1,824	IV	"
6	32	9/2/65	—	III	
		21/7/65	—	IV	No. Punch biopsy
		12/10/65	Nil	IV	negative 19/3/65
		11/11/65	"	III	
7	47	4/3/65	394	III	
		10/6/65	Nil	IV	Yes
8	32	30/3/65	"	III	
		30/11/65	"	IV	"
9	30	6/4/65	"	IV	
		13/4/65	"	IV	"
10	57	14/4/65	150	IV	
		5/5/65	Nil	IV	"
11	53	11/5/65	"	IV	
		25/5/65	94	IV	"
12	34	20/5/65	Nil	III	
		3/6/65	—	IV	No. Negative
		6/1/66	—	III	biopsy 30/7/65
13	44	25/5/65	32	IV	
		17/8/65	37	IV	Yes
14	39	3/6/65	Nil	IV	
		17/6/65	"	IV	
		29/7/65	8	IV	
15	28	8/7/65	Nil	III	
		17/8/65	"	III	
		11/11/65	—	IV	
		Hospital Patients			
16	42	13/10/64	Nil	IV	"
		28/10/64	"	IV	
17	30	13/5/65	24	II	No biopsy
		18/11/65	—	I	
18	36	24/11/64	Nil	IV	Yes
		7/11/64	3	IV	
19	47	2/12/64	409	II	
20	26	10/12/64	Nil	—	
		4/2/65	—	IV	
		13/5/65	Nil	—	
		31/8/65	—	IV	
21	25	5/2/65	12	III	
		28/6/66	—	IV	
22	36	11/2/65	43	III	
		29/4/65	Nil	III	
		29/5/65	"	III	
23	45	18/2/65	—	Unsatisfactory smear	
24	37	12/4/65	—	IV	
		3/5/65	—	IV	
		13/5/65	152	—	
25	33	26/4/65	—	IV	
		26/7/65	52	IV	
		9/8/65	129	IV	
		23/8/65	58	IV	
26	37	3/5/65	—	III	
		14/6/65	—	IV	
		21/6/65	58	—	
27	31	20/5/65	Nil	III	
		17/6/65	—	III	
		1/7/65	—	III	
28	48	21/5/65	146	IV	
		9/7/65	—	IV	
29	24	15/7/65	Nil	III	
		12/8/65	—	III	
		24/2/66	—	IV	
30	26	2/8/65	Nil	III	
		18/1/66	—	IV	
31	46	31/8/65	—	IV	
		4/9/65	—	IV	
		10/9/65	62	—	

histological examination demonstrated "a transitional zone showing basal hyperactivity, which . . . is of sufficient degree to amount to an early carcinoma-in-situ." In a further case (No. 23) the initial smear was reported as unsatisfactory but was not repeated. Subsequent biopsy on clinical grounds (the patient complained of postcoital bleeding) demonstrated a carcinoma-in-situ.

TABLE IX.—*Enzyme and Cytology Results Relating to Invasive Carcinomas*

No.	Age	Date	Enzyme Result (Units)	Cytology Grade	Diagnosis
			Practice Patient		
32	50	7/4/65	300	III	Carcinoma of cervix
		26/5/65	110	III	
		24/8/65	—	III	
			Hospital Patients		
33	61	6/11/64	6	IV	Adenocarcinoma of corpus
		23/11/64	463	—	
34	56	13/11/64	382	III	,, ,,
35	71	3/2/65	387	IV	,, ,,
36	59	10/2/65	Nil	IV	,, ,,
37	43	7/5/65	148	IV	Secondary carcinoma of cervix from primary in bronchus
38	49	16/7/65	32	IV	Carcinoma of cervix
39	50	9/8/65	185	IV	,,
40	57	20/8/65	—	IV	,, ,,

Invasive Carcinoma Data.—Two adenocarcinomas of corpus (Cases 33 and 36) and one carcinoma of cervix (Case 38) each had one false-negative enzyme estimation.

"False" Positives

If we accept cytology grades I and II as presumptive evidence of freedom from cervical cancer, and use again the arbitrary level of 100 units and over as denoting a positive enzyme test, it is possible to estimate false-positive rates from the data in Table VII. Thus among 3,439 women cytologically graded I and II there were 535 (16%) with estimated 6-phosphogluconate dehydrogenase levels of 100 units and over. This proportion of false positives was higher (23%) among women graded II in cytology than among those graded I (11%). Separate consideration of data relating to premenopausal and postmenopausal women indicated that the percentage of false-positive enzyme results was higher among the latter group. Thus while 12% of premenopausal patients, graded I and II on cytology, had enzyme results of 100 units or more, this proportion rose to 33% among postmenopausal women. In both groups the relation with cytology grading remained apparent with, for premenopausal patients, a false-positive rate of 9% associated with grade I cytology, rising to 18% for grade II, and a comparable increase from 27 to 47% among postmenopausal women.

Among 26 patients with positive smears, two (Cases 6 and 12) had a biopsy which failed to confirm the cytological diagnosis, and a further patient (Case 17) had an initial grade IV cytology which reverted to normal and biopsy was not performed. Thus the proportion of false-positive cytologies has been 2 out of 26 (8%), or 3 out of 26 (12%) if Case 17 is included in this category.

Enzyme Results Related to Age, Menstrual Cycle, and Menopause

A distribution by age of enzyme estimations from the 3,928 patients for whom results were available indicated that enzyme levels were higher among older women (Table X). The proportion of women between 20 and 39 years with levels above 300 units varied from 3 to 6%. Among older women comparable proportions rose steadily from 7% at 40–44, to 9% at 45–49, to 15% at 50–54, and to 24% at 55–59 years. Similarly, while 67% of women aged 25–29 years had completely negative results—that is, nil units—this percentage showed a fall in each succeeding five-year age group until at 60 years and over only 32% of patients had completely negative enzyme tests.

Further consideration of the age-specific data by type of clinic attended suggested a difference in enzyme levels between pregnant women attending the hospital antenatal clinic and other survey patients (Table XI). It was apparent that these antenatal patients had yielded both a higher proportion of negative results and a smaller proportion of high enzyme values (100 units+) than women of the same age attending either the remaining hospital or practice clinics. In contrast, distributions of enzyme levels among hospital patients other than antenatal and among practice patients were closely similar at all ages up to 60 years. It appeared that in both the latter groups the main increase in enzyme level with age occurred between the fifth and sixth decades. It seemed, therefore, that the overall age gradient apparent in Table IX stemmed, at least in part, from the contribution of antenatal patients with their generally lower enzyme level to the youngest age groups. Among other patients the pattern of enzyme distribution showed little variation with age until the sixth decade, when there was a marked increase in frequency of high values.

Results of enzyme estimations for premenopausal women were analysed separately by time of collection—that is, day of cycle—of specimens (Table XII). Though specimens were not usually taken during menstruation, hence the smaller number of patients with data relating to first week of cycle, it remains possible that contamination of specimens may have been a factor tending to distort the distribution of results during this first week of the menstrual cycle. A similar factor may also have been responsible for the relatively higher enzyme values among the few (33) postnatal patients included in the survey. Otherwise, the data provided no evidence of any relation between enzyme level and the time of collection (day of cycle) of specimen. In each of the four groups studied (1–7, 8–14, 15–21, and 22+ days) some 14–15% of specimens gave results of 100 units or more.

TABLE X.—*Distribution of Enzyme Results by Age*

Age (Years)	Enzyme Results (Units)												Total
	Nil		1–49		50–99		100–199		200–299		300+		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Under 20	13	*68*	6	*32*	—	—	—	—	—	—	—	—	19
20–24	302	*65*	101	*22*	26	*6*	16	*3*	7	*2*	13	*3*	465
25–29	510	*67*	132	*17*	34	*4*	37	*5*	17	*2*	30	*4*	760
30–34	389	*61*	128	*20*	40	*6*	28	*4*	15	*2*	35	*6*	635
35–39	322	*57*	117	*21*	56	*10*	27	*5*	11	*2*	27	*5*	560
40–44	298	*59*	105	*21*	29	*6*	29	*6*	11	*2*	35	*7*	507
45–49	197	*53*	82	*22*	25	*7*	20	*5*	17	*5*	32	*9*	373
50–54	148	*48*	55	*18*	21	*7*	27	*9*	12	*4*	48	*15*	311
55–59	66	*40*	22	*13*	11	*7*	15	*9*	13	*8*	40	*24*	167
60+	49	*38*	17	*13*	17	*13*	7	*5*	12	*9*	26	*20*	128
N.K.													3
Total ..	2,294		765		259		206		115		286		3,928

TABLE XI.—*Proportional Distribution of Enzyme Results by Age and Clinics*

Age (Years)	Proportion of Patients (%) with given Enzyme Results (Units)				Total Patients
	Nil	1–49	50–99	100+	
Hospital Clinics (Antenatal)					
Under 20	57·1	42·9	—	—	14
20–29	70·7	18·5	4·7	6·1	676
30–39	68·6	19·3	6·2	5·9	357
40–49	56·5	21·7	6·5	15·2	46
Hospital Clinics (Other than Antenatal)					
Under 20	100·0	—	—	—	5
20–29	60·6	20·7	5·7	13·0	246
30–39	52·4	21·6	10·7	15·4	338
40–49	57·2	21·0	6·8	15·0	353
50–59	45·3	16·8	7·5	30·4	161
60+	45·2	16·7	7·1	31·0	42
N.K.					2
Practice Clinics					
20–29	61·1	18·8	4·6	15·5	303
30–39	57·8	20·6	7·6	14·0	500
40–49	55·5	21·4	5·6	17·5	481
50–59	44·5	15·8	6·3	33·4	317
60+	34·9	11·6	16·3	37·2	86
N.K.					1

TABLE XII.—*Proportional Distribuion of Enyzme Results Among* 3,295 *Premenopausal Women by Day of Cycle*

Day of Cycle	Proportion of Patients (%) with given Enzyme Result (Units)				Total Patients
	Nil	1–49	50–99	100+	
1–7	44·1	25·0	15·8	15·1	152
8–14	59·7	20·3	6·4	13·6	675
15–21	57·9	20·7	6·0	15·3	632
22+	58·7	19·7	6·6	15·1	651
N.K.	65·8	13·2	7·9	13·2	38
Pregnant	69·1	19·5	5·0	6·4	1,114
Postnatal	39·4	27·3	9·1	24·2	33

The greater frequency of high enzyme values among postmenopausal patients has already been noted. A more detailed examination of results among these women revealed a consistent increase in yield of high enzyme results with time since the menopause (Table XIII). Levels of 100 units or above occurred among 28% of women who had reached the menopause 0–4 years before entry to the survey. This proportion rose successively to 35% and 40% among patients with time intervals since menopause of 5–9 years and 10–14 years respectively, while more than half of the group with a postmenopausal experience of at least 15 years had enzyme levels of above 100 units. This trend of increasing high values with time since the menopause was accompanied by a comparable decline of negative and low enzyme levels over the same time scale.

TABLE XIII.—*Proportional Distribution of Enzyme Results Among* 633 *Postmenopausal Women by Years Since Menopause*

Years since Meno-pause	Proportion of Patients (%) with given Enzyme Result (Units)				Total Patients
	Nil	1–49	50–99	100+	
0–4	47·5	18·1	6·7	27·6	326
5–9	41·6	16·1	7·3	35·0	137
10–14	33·7	13·0	13·0	40·2	92
15+	32·4	8·1	8·1	51·4	74
N.K.					4

Enzyme Results Related to Other Findings

Analysis of enzyme data in relation to features routinely recorded for survey patients demonstrated that results were notably higher among patients with concurrent *Trichomonas vaginalis* or atrophic vaginitis. Only 39 (23.6%) of 165 women with trichomonas infestation had completely negative enzyme results, 26 (15.8%) had enzyme values of 1 to 49 units, and 13 (7.9%) had values in the 50–99 units range. The remaining 87 (52.7%) had enzyme results of 100 units or more. The distribution of enzyme estimations among patients with atrophic vaginitis was also shifted upwards, the combined experience of 102 hospital and practice patients showing 31.4% with completely negative results and, at the other end of the scale, 44.1% of patients with enzyme values of 100 units and over. Among patients with other concurrent findings the results were less striking. These results are summarized in Table XIV, which presents proportions of high enzyme values (>100 units) among patients, by clinic, for each of the concurrent findings listed. Apart from patients with trichomonas infestation or atrophic vaginitis, and considering only those proportions based on at least five patients, the three conditions associated with a two-fold increase in the proportion of high enzyme values (>100 units), compared with that among patients with normal findings on vaginal examination, were vulvitis (practice patients), other vaginitis (hospital patients other than antenatal), and irritating vaginal discharge (antenatal patients).

TABLE XIV.—*Proportions of High Enzyme Results* (100 *Units and Over*) *Related to Concurrent Findings*

Concurrent Findings	Proportion* of Enzyme results (%) >100 Units among Patients Attending		
	Antenatal Clinics	Other Hospital Clinics	Practice Clinics
On vaginal examination:			
Normal	5·4	14·8	18·1
Vulvitis	(25·0)	(25·0)	39·7
Atrophic vaginitis	—	51·7	41·1
Other vaginitis	(11·1)	34·5	27·9
Erosion	7·5	18·8	18·6
Polyp	—	18·0	23·1
Chronic cervicitis	(6·7)	18·8	16·7
From patient's history:			
Vaginal discharge	7·2	18·3	19·8
Irritating vaginal discharge	11·0	20·0	23·9
Intercourse within 48 hours (semen entering vagina)	6·2	12·5	14·3
Douche within 48 hours	(11·8)	14·3	23·3
Disinfectant in bath	8·8	19·0	23·0
On cytological examination:			
Trichomonas vaginalis	27·3	52·7	61·9

* Proportions in parentheses based on fewer than 5 cases.

Discussion

An acceptable biochemical test would be a welcome addition to established methods of population screening for cervical cancer. Compared with cytology, a biochemical approach might be expected to be less demanding on available resources and easier to carry out on a mass scale, but its practical acceptance must depend on a demonstration of ability to match the performance of cytological methods, particularly in the detection of preinvasive lesions and early carcinomas.

False-negative Enzyme Results

The first suggestion that 6-phosphogluconate dehydrogenase activity in vaginal fluid might provide a new enzyme test for gynaecological cancer (Bonham and Gibbs, 1962) was based mainly on results from clinical cases. Three cases only of carcinoma-in-situ, all with positive enzyme tests, were included in the study. In the present survey 21 (78%) of 27 patients with confirmed preinvasive lesions had at least one negative enzyme result (<100 units/g. dried weight), and among 15 cases with more than one enzyme estimation 10 (67%) were consistently negative by the same standard. Three other studies have also reported varying proportions of false-negative enzyme results among patients with carcinoma-in-situ—2 out of 3 (Muir *et al.*, 1964), 5 out of 11 (Cameron and Husain, 1965), and 5 out of 6 (Bell and Egerton, 1965). Combining the experience of these five investigations provides a total of 50 carcinoma-in-situ cases, of which 33 (67%) have been associated with a negative enzyme test. It is clear, therefore, that the test in its present stage of development does not offer a practical alternative to cytology for detecting the histological entity of carcinoma-in-situ.

With respect to invasive cervical carcinoma, the absence of false-negative enzyme results among 26 cervical cancers (Bonham and Gibbs, 1962) has been confirmed in a further series of 40 cases (Cameron and Husain, 1965). On the other hand, one of three primary carcinomas of cervix in the present study had an enzyme result of 32 units, and Lawson and Watkins (1965) reported five false negatives among 49 cases of cervical cancer.

False-positive Enzyme Results

False-positive enzyme (6-phosphogluconate dehydrogenase) results among women without malignant disease have varied between 3% (Bonham and Gibbs, 1962) and 47% (Lawson and Watkins, 1965), a variation partly due to differences between centres with respect to assay method and the nature of the population studied. In the present investigation 16% of 3,439 women with no cytological evidence of malignancy had enzyme levels of 100 units and above, a false-positive rate in close agreement with that found among patients attending a well-women clinic (Muir *et al.*, 1964). Enzyme levels were notably low, as measured by a false-positive rate of 6%, among women attending the hospital antenatal clinic, and it appeared that this favourable experience of antenatal patients was not simply a reflection of their relative youth compared with patients attending other hospital and practice clinics (Table XI). Enzyme distribution in the two latter groups were similar; an enzyme activity of 100 units or more was recorded for about 15% of women aged 20–59 years, and this proportion increased to over 30% among women in the sixth and seventh decades.

Previous indications that incidence of false-positive enzyme reactions among premenopausal women is unrelated to the phase of menstrual cycle in which specimens are taken, and that there is a marked increase in high enzyme values among postmenopausal women (Cameron and Husain, 1965) have been confirmed. Women in the latter group showed steadily increasing enzyme levels with time since menopause, and more than half of those with a postmenopausal experience greater than 15 years had enzyme results above 100 units. High 6-phosphogluconate dehydrogenase activities were also notably frequent among patients with concurrent trichomonal infection or atrophic vaginitis and, to a less extent, among women with other signs or symptoms of infection.

Relevance of Natural History of Preinvasive Lesions

The failure of the 6-phosphogluconate dehydrogenase enzyme test to pick up a large proportion of preinvasive lesions has now been confirmed in several studies. In our present state of knowledge it would clearly be unethical to leave these patients untreated, and the test cannot be regarded as a practical alternative to cytological examination. It may be, however, that a final assessment of the role of biochemical tests in cervical screening must await further clarification of the natural history of carcinoma-in-situ. Since the certain diagnosis of this well-established histological entity is itself destructive, it follows that histological studies *per se* cannot define its natural history—for example, how long carcinoma-in-situ lasts, how often it resolves, how often it progresses. The more optimistic hopes for cervical screening—for example, that it represents a means of eliminating cancer from a population (Younge, 1965)—are based on the assumption that all invasive carcinomas pass through an in-situ phase which can be detected by cytological screening.

A different view has been taken by Ashley (1966a, 1966b), who has suggested that there may be two biologically different forms of cervical carcinoma. The first of these types is characterized as slow-growing, susceptible to treatment, occurring in younger women, and likely to pass through an in-situ phase, while the second is more rapidly growing, is more resistant to treatment, and occurs later in life without a preceding carcinoma-in-situ. Implicit in this hypothesis were the conclusions that extensive cytological screening of the female population was unlikely to eliminate cervical carcinoma as a cause of death, and that management of carcinoma-in-situ could be conducted on conservative lines.

A recent re-examination of the evidence (Knox, 1966) has also found no conclusive support for widely held assumptions associated with mass screening and has emphasized the urgent need for a definitive answer to what remains the central problem in this field—that is, the natural history of preinvasive lesions. Knox presents a reasoned argument suggesting that solution of the problem may require a well-planned population study involving the serial screening of around 100,000 women over a period of five to six years. In the absence of conclusive data it is perhaps not surprising that estimates of the proportion of carcinomas-in-situ which progress to invasive carcinoma have varied from 30% (Petersen, 1955) to 60% (Boyes *et al.*, 1962), and until this question is settled it would appear that speculation about a possible role for a biochemical test in differentiating carcinoma-in-situ which progress from those which do not must remain inconclusive.

Practical Difficulties of Population Screening

The experience of general practice clinics obtained from the present study has also reaffirmed some of the practical difficulties of population screening—for example, the contrast between popular demand for such services and the relatively low acceptance rates when they are offered freely. While spontaneous individual responses to the survey's introductory letter were almost invariably favourable, less than half of the women eligible finally attended the clinics. Among stated reasons for non-attendance, fear of cancer or dislike of the examination was directly expressed by 25% of patients, and it is possible that similar fears may have been shared by some of the further 40% of non-responders who volunteered only vague indefinite reasons for not attending.

The problem of indifferent response is accentuated by the differentially low acceptance of screening among high-risk groups (Macgregor and Baird, 1963). In the present survey it was notable that the practice (B) with a social class distribution most heavily weighted with classes IV and V had the lowest response rate (42%) and highest yield of positive cases (10.2 per 1,000). Fear of cancer and dislike of examination were also expressed most commonly by non-attenders from this practice. In contrast, the practice (C) with the highest proportion of patients in social classes I and II had the most favourable response (62%) and lowest prevalence rate (2.6 per 1,000). This pattern of response clearly suggests that any effective development of community screening may well require a major campaign directed toward acceptance of facilities, particularly among the less health-conscious women at greatest risk.

While from the community point of view a comprehensive service implies one in which all women at risk are examined repeatedly at regular intervals (Lawson, 1966), the establishment of such a service must, on any assessment of present resources, be regarded as a future rather than immediate prospect. In this situation Jeffcoate (1966) has suggested that a reasonable coverage of women might be achieved by introducing cervical cytology as a routine for all patients attending a range of gynaecological and antenatal clinics. With resources currently available, it can be argued that this represents a realistic approach and the one best fitted to meet practical demand in the immediate future. A routine service based on hospital (and practice) clinics is, however, unlikely to provide answers to present doubts and uncertainties concerning the ultimate role of mass screening in detecting and preventing cervical cancer. A well-planned population study might be expected to do so

(Knox, 1966) and provide a firmer base for decision on long-term planning and development. Thus whatever approach is made to immediate provision of services, the more intensive local study of communities large enough to produce definitive answers (Lawson, 1966) remains an essential component of any general screening programme.

Summary

Comparison has been made between biochemical (6-phosphogluconate dehydrogenase activity) and cytological methods of cervical screening in a survey of 4,385 married women attending hospital and general practice clinics. The survey detected 28 cases of carcinoma-in-situ, an overall yield of 6.4 per 1,000. Among the 27 patients for whom enzyme assessment was available, 21 (78%) had at least one result showing an enzyme activity of less than 100 units/g. dried weight. Among 15 carcinoma-in-situ patients with more than one enzyme result, 10 (67%) were consistently negative by the same standard. With such a high proportion of false-negative results the enzyme test, as at present developed, cannot be regarded as a practical alternative to cytology for detecting preinvasive cervical cancer.

The study's experience of general practice clinics confirmed the contrast between popular demand for cervical screening and the relatively low acceptance rate when this service is offered freely. Less than half of the women eligible attended the clinics. Study of response and prevalence rates from individual practices in relation to their respective social class distribution supported the view that acceptance of screening is lowest among the less health-conscious women at greatest risk, and that this differential response represents a major problem in the development of any effective comprehensive service. The more optimistic hopes for such a service have yet to be realized, and, whatever approach is made to immediate provision of screening facilities, doubts and uncertainties which remain point to the need for supplementing any general programme with more intensive population studies at a local level.

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Isoniazid Therapy in Relation to Later Occurrence of Cancer in Adults and in Infants

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This study was initiated as a result of a communication in the *British Medical Journal* by Roe, Boyland, and Haddow (1965), who pointed out that in the light of experimental evidence there was an urgent need for epidemiological studies on the possible carcinogenic effects of isoniazid therapy. More recently, similar sentiments were expressed in a strongly worded editorial in the *Lancet* (1966).

Difficulty with the epidemiological approach at this time lies in the fact that known carcinogenic agents rarely produce cancer in adult human beings until about 10 to 20 or more years after first exposure. Isoniazid was first administered experimentally to two men (I. J. S. and E. H. R.) in June 1951 and therapeutically to a sizable number of patients with tuberculosis at Sea View Hospital shortly thereafter (Selikoff, Robitzek, and Ornstein, 1952). Less than 16 years have elapsed since that time.

On the other hand, some carcinogenic chemicals act very rapidly when administered in utero to experimental animals (Druckrey, Ivanković, and Preussmann, 1966), and whatever factor or combinations of factors cause cancer in children must operate in a relatively short time. For example, in New York State in the period 1958–60 the highest incidence rate of childhood leukaemia (9.2 per 100,000 a year for males and females combined) occurred in 4-year-old children, and the rate was appreciable (3.7 per 100,000) even in 1-year-old children (Ferber, Handy, Gerhardt, and Solomon, 1962). For childhood cancer of all types and sites combined, the highest incidence rate (16.4 per 100,000) occurred in 4-year-olds, but the rate was almost as high at ages 1, 2, and 3 years.

Since certain hormones have an influence on the growth rate of cancer, it is conceivable that pregnancy might enhance the potential carcinogenicity of isoniazid.

Over the years some authors have suggested that tuberculosis increases the risk of lung cancer, while others have suggested exactly the opposite. Further information on this point is pertinent to the problem at hand.

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