Patients in group 1 are treated by pain relief alone. If this is not sufficient to maintain adequate ventilation, they require tracheotomy and fall into group 2. All those who still cannot ventilate adequately need I.P.P.R. and fall into group 3.

The maintenance of the treatment appropriate to each group is discussed, and it is emphasized that frequent assessments of ventilation should be made in order that deterioration shall not go unrecognized.

Pain can be completely controlled by thoracic segmental extradural block, and it is felt that the use of this technique decreases the need for tracheotomy or I.P.P.R., especially in the elderly and those with pre-existing chest disease.

Illustrative case reports are presented. The overall mortality in the series is 9.09%.

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Cytological Diagnosis of Cancer: Its Uses and Limitations

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The diagnosis of neoplastic disease still rests on the skilled interpretation of microscopical appearances. Although an empirical procedure, the examination of sections from a sufficiently large piece of tissue nearly always permits a trained observer to state correctly whether there is a malignant tumour or not. If tongues of neoplastic epithelium are seen growing down into the normal tissue of an organ, this may furnish a very convincing demonstration; but the histologist is often able to make a reliable diagnosis from a piece of solid tumour in which there is no normal tissue left to be invaded. He does this by an experienced judgment of the morphology of the cells and their arrangement relative to each other and to the stroma.

A diagnosis of malignancy made by examining smears of exudates, natural secretions, and scrapings is in general less reliable than one given by the histopathologist. But if a cytologist soberly bases his opinions on a sufficiently large past experience, and does not attempt inferences from appearances with which he is not familiar, he is able to detect malignancy in cases which otherwise would be missed, and sometimes to make a firm diagnosis in cases where the tumour is inaccessible to biopsy.

Now that the demand for cytological diagnosis of cancer is becoming more insistent many pathologists are put in the position of providing a service without feeling very sure of the value of it. In some centres cytodiagnosis has been used in one of its applications only, and the person responsible is in doubt whether to extend his service from, for example, sputum diagnosis to examine cervical smears or gastric washings as well. The literature on the whole consists of enthusiastic reports, and one does not always know whether to expect such good results in conditions of routine application.

This paper is intended to give a brief guide to what in our opinion are the most important applications of cytodiagnosis. It is based partly on personal experience and partly on an examination of the literature.

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The cytological method has two different fields of usefulness in clinical pathology. First, it can be used as a *detection* test for screening populations. When applied in this way it is only important that no case should slip through the net; accuracy of diagnosis is immaterial, as all suspicious cases will be fully investigated subsequently. Secondly, the cytological method may be used to give a definite *diagnosis*. In this case it is of vital importance that "positive" reports should be accurate; the observer is giving an opinion that a patient has malignant disease, and if the diagnosis later turns out wrong this is a serious mistake. When cytological reports are given it should be made quite clear whether the object is detection or diagnosis.

At Oxford the cytological method has been used for both these purposes. In the past 12 years 28,874 specimens have been examined for the detection of lesions of the female genital tract, and 22,609 specimens of various kinds for diagnostic purposes.

For the first five years all smears were scanned by one of us (mostly by M. M. B.), then one, two, and recently three technicians were employed. Since research programmes were undertaken concurrently, it is not possible to calculate from this the number of smears which can reasonably be examined by one person per day. McLaren (1963) suggests 30 per day as being within the limits set by fatigue, but in the early years of this series this was sometimes far exceeded. In the U.S.A. the number examined per technician per year is between 3,000 and 5,000 (Horn and Siegel, 1961).

Method of Reporting

We have used a flexible system of wording rather than the five classes of Papanicolaou. Where the object of the test is detection (cervical smears) there is no need for a categorical

diagnosis of "malignant cells" (which in the event often come from pre-invasive lesions). The smears are either suspicious and an indication for biopsy or they are not.

Where the examination is for diagnostic clarification we determine our system of reporting on the degree of confidence felt as based on the "false-positive" figures. In the case of sputum, certain common appearances carry only a 95% certainty of carcinoma, and we report most of these as "highly suspicious." When the false-positive rate is negligible, as with serous effusions, we feel free in many cases to speak of "malignant cells" being found.

Cervix Uteri

The vaginal or cervical smear provides the outstanding example of a detection test. By means of these procedures it is possible to pick out precancerous or very early malignant lesions of the cervix with high sensitivity, and permit treatment to begin at a stage where the expectation of genuine cure approaches 100%.

There is very good evidence now for regarding "carcinoma-in-situ" as a precancerous condition. Even when subjected to repeated biopsy, about 30% of these lesions have been shown to progress eventually to invasive carcinoma (Petersen, 1955); and evidence from the difference between the incidence of carcinoma-in-situ and of invasive carcinoma suggests that 60% of untouched carcinomas-in-situ progress to invasion (Boyes *et al.*, 1962). It is therefore very likely that more than half of the cases picked up by the screening method are actually saved from developing cervical cancer. An almost 50% reduction in the incidence of this disease has been achieved in British Columbia, where 50% of women aged over 20 have been screened (Bryans *et al.*, 1964).

Numerous large series of cases reported from all over the world have given an essentially uniform picture of the results expected in "screening" new subjects by any of the principal variations of the method.

In large series of patients screened for the first time the prevalence of carcinoma-in-situ is about 4 per 1,000, and of unsuspected invasive carcinoma about 2 per 1,000 (Day, 1961). Figures showing the rates among patients being re-screened at intervals of at least a year have been given, among others, by Erickson *et al.* (1956), Dunn *et al.* (1959), and Christopherson *et al.* (1962). These show a tenfold reduction in the numbers of invasive carcinoma and a lesser fall in the numbers of carcinoma-in-situ. This demonstrates a large difference between prevalence and yearly incidence of carcinoma of the cervix, proving that it has a long preclinical course and is susceptible to elimination following early diagnosis.

The Oxford series consists of smears taken by the gynaecologists using the Ayre spatula, and from 1952 to 1963 the tests were done routinely on gynaecological out-patients only. The Ayre scrape was chosen as the sole method because it appeared to be the most sensitive technique for cervical lesions. The Papanicolaou staining method has been used throughout.

Reports are given in the form of a brief description of the cells, together with a recommendation to biopsy or to repeat if necessary.

Our results for the first seven years were published in 1960 (Boddington *et al.*, 1960). The series followed up now consists of 23,263 smears from 18,000 patients, and the yield of cases whose biopsies showed carcinoma, carcinoma-in-situ, or dysplasia is shown in Table I. A total of 129 *unsuspected* cases of malignant or probably precancerous lesions have been found.

The distinction between carcinoma-in-situ and dysplasia depends on the opinion of the histologist examining the specimen, and is not particularly important if both conditions carry a risk of developing into cancer. This vexed question is the subject of a voluminous literature; the reader is particularly referred to the papers of Petersen (1955), and Koss *et al.* (1963), and to the books by Fluhmann (1961) and Gray (1964).

TABLE I.—*Results of Cervical-smear Examination of* 18,000 *Cases** (1952–63)

Final Diagnosis	Cytologically Suspicious	Cytologically Negative
Carcinoma of cervix	154	14
Carcinoma-in-situ and dysplasia of cervix ..	133	14
Carcinoma of endometrium	34	87
No carcinoma dysplasia	59	17,432

* 73 cases are omitted because they do not fit in the above simplified table. These are: 33 with abnormal smears but lost to follow-up; 29 carcinoma of vulva or ovary with correct suspicious smears; 10 with carcinoma of the genital tract with abnormal smears but in which no definite opinion whether suspicious or negative was given; and one case with a suspicious smear finally diagnosed as carcinoma-in-situ of the endometrium.

A word should be said about the accuracy of the cervical smear in carcinoma of the cervix, expressed in numerical terms. Although many authors give a figure for "false positives," this is meaningful only in conditions where the histologist (whose opinion is final) can answer yes or no. Since the object of the test is to discover abnormalities which are extremely variable and, in any given case, of unknown prognosis, it is impossible to say which lesions discovered by the cytologist are right and which are wrong. When no histological abnormality is found at all, this probably means that the affected area has not been included in the sections. In our series no significant lesion was found in 38 cases, or 12% of cases in which biopsy of the cervix was advised. (The figure of 59 in Table I includes 21 cases in which carcinoma of the endometrium was suspected.)

Turning to false-negative reports, it is obviously of great importance that these should be as few as possible. Unfortunately the size of this category of errors is unknown, because nobody performs biopsies routinely on patients with negative cytological reports. Most of the cases of carcinoma of the cervix in Oxford become known to us, and those which have negative smears are recorded; in this way it is possible to say that 14 were missed, or 8% of all cases of carcinoma of the cervix. The true figure including carcinoma-in-situ must be larger than this.

False negatives are given by von Haam (1962) as 13%, by Graham (1963) as 3.3%.

Endometrium

Although occasionally it is possible to suspect endometrial carcinoma from cervical smears, the methods recommended for the purpose are the original vaginal-smear method of Papanicolaou and the endometrial aspiration smear.

Cytological methods have not proved very useful in this condition. The principal reason is that the range of appearances of uterine adenocarcinoma cells overlaps considerably with that of non-malignant endometrial cells. Most authorities state that the distinction is "difficult," meaning (one may guess) that in many cases it cannot be made at all. According to Koss and Durfee (1961), "it is not advisable to diagnose endometrial abnormalities in vaginal smears obtained during the first 10 days of the cycle, nor is it advisable to draw any conclusions concerning the endometrium from cervical smears." As for endometrial aspirations, these are clearly inapplicable to mass screening, while for diagnostic purposes a curettage will be preferred.

If vaginal smears are employed the false-negative rate for endometrial carcinoma has usually been given between 16 and 30%. The false-positive rate has generally been high; but was only 5% according to Graham (1963).

In our series of cervical smears, in which endometrial carcinoma cells are found only accidentally, there have been 34 cases reported as suspicious of carcinoma among 123 cases of endometrial carcinoma studied. There is therefore a false-

negative rate of 72%, which is unacceptable for a detection technique. During the same period 21 smears were erroneously reported as suspicious of endometrial carcinoma. (It must be emphasized that in a detection test a "positive" report is an indication for biopsy and not an opinion about the significance of the cells.) These results justify Koss's remark quoted above, and we are in a dilemma whether or not to report a suspicion of carcinoma of the body based on the morphology of the endometrial cells.

Unsuspected cases of endometrial carcinoma can sometimes be picked up from vaginal or cervical smears by observing features other than morphological evidences of malignancy in the cells. The presence of endometrial cells (whether normal or not) after the menopause is an indication for a diagnostic curettage.

Lung

In spite of the long period required for the development of carcinoma of the bronchus (as shown in the statistics relating to tobacco smoking), it is a disappointing fact that cytological methods have contributed very little to early diagnosis of this disease. Precancerous lesions of the bronchi (carcinoma-in-situ) are hardly ever found during life, and their incidence in post-mortem material is not generally agreed to be high. There are a few reports of very early malignant lesions, some of them discovered by sputum examination (Papanicolaou and Koprowska, 1951 ; Woolner *et al.*, 1960 ; Mavrommatis, 1962 ; Melamed *et al.*, 1963), but the screening of symptom-free persons is not usually considered worth while. Rome and Olson (1961) screened 776 men without detecting any case of carcinoma of the lung.

For purposes of diagnosis, as opposed to detection, cytological examination has been in use for many years and is regarded as a valuable procedure (Grunze, 1960 ; Koss *et al.*, 1964). Full details of technique and interpretation are given by Farber *et al.* (1950) and Koss and Durfee (1961). Our practice has been to receive samples of unfixed sputum—three specimens from each patient if possible. Two slides are made from each of two separate chosen samples of sputum, making four in all per specimen. They are fixed in ethyl alcohol containing 3% of acetic acid, and are stained by Papanicolaou's method. Normally only one slide is examined from each pair, but all four are used in cases of doubt.

In the period under review we have examined sputum or bronchial aspirates from 6,372 patients. The reports have been worded to express the degree of confidence felt in the diagnosis: "highly suspicious of squamous carcinoma," "typical of oat-cell carcinoma," "numerous anaplastic tumour cells present," and so on. For statistical purposes we classify all these as "positive, and they correspond to Papanicolaou's classes III, IV, and V or the "suspicious" and "positive" classes of other authors. In this laboratory a report of "suspicious" gives the clinician to understand that evidence of malignancy has been found.

In the whole series 936 "positive" reports were given, and 918 of these were confirmed or accepted as correct on other grounds. Of 27 "positive" reports believed to be false, nine were subsequently proved correct after intervals of one year (3 cases), two years (4 cases), three years (1 case), and four years (1 case). The remaining 18 cases are regarded as false positives (false Papanicolaou III, IV and V) and amount to 1.9% of all "positive" reports. It is because of this proportion of mistakes (which the literature suggests is irreducible) that we prefer to use the words "highly suspicious" in most cases even when hardly any doubt exists.[1]

The incidence of false-negative reports can be calculated only if all cases are followed up, including those reported "negative." We obtained this information for the years 1952–7 and found that of 885 cases of bronchial carcinoma having sputum examination 453 (52%) were given positive reports. This includes all cases even if only one or two samples were sent. When three were sent the proportion of positives rose to 63%. The adoption of better standards (particularly in insisting on genuine sputum with little salivary contamination) probably explains how some laboratories have achieved a very much higher rate of success, sometimes exceeding 85%. Russell *et al.* (1963) have presented a histogram (their Fig. 13) relating the percentage of positive diagnoses to the number of specimens submitted. This rises from 23% with one or two specimens to 77% with 10 or more.

Several authors have found equally high positive rates among cases with resectable tumours as among the inoperable ones, and we found this in our 1957 series (Spriggs, 1957a), but it has not been the universal experience (Duguid and Huish, 1963).

Bronchial aspirates have been accepted for cytological examination in this laboratory, but for several reasons we have not found them so satisfactory as sputum. There is usually only a single specimen which cannot easily be repeated. If the cells are suspended in saline they deteriorate very rapidly after removal, whereas sputum is still fit for examination after a day's delay. Fixation immediately after aspiration has been of no advantage because it increases still further the tendency to shrinkage and overstaining of the cells, which is very often troublesome in bronchial aspirates. In order to obtain good results with this material it appears essential that a cytologist or technician should be present at bronchoscopy to see that the slides are made on the spot.

Cytological diagnosis in the confirmation of carcinoma of the lung is already so well established that there is a tendency to use it excessively. If this examination is to be done properly it is time-consuming—nearly an hour's microscopy will usually be needed in order to declare one case cytologically negative on the basis of three samples—so that it should be performed only when a positive answer would be clinically useful. When a patient is admitted to a thoracic surgery ward it is better to collect and process samples for subsequent examination if other investigations are inconclusive. If the treatment for cancer is planned and carried out before the cytological report is received, or in spite of a negative one, then the cytologist's time has been wasted.

Gastro-intestinal Tract

Mouth

We have no personal experience of the diagnosis of carcinoma from buccal smears. This method probably has little place in presymptomatic diagnosis in Western countries, owing to the rarity of oral carcinoma. In the series of Selbach and von Haam (1963) nearly half of the cases of carcinoma were given a class III (suspicious) report (40 out of 93), and a class III report was also given in 6 out of 32 cases of leucoplakia and in 6 out of 167 cases with entirely benign lesions. Sandler (1964) also shows a large "doubtful" and "suggestive" category. It is apparent that there is a large overlap between the appearances of benign and malignant cells.

Oesophagus

Again we have hardly any personal experience of this site, but the value of the method for diagnosing carcinoma of the oesophagus can be gauged from the literature.

In the large series of Johnson *et al.* (1955) oesophageal washings were examined from 364 patients, including 148 with malignant tumours of the oesophagus. Of the latter, 103 were reported "positive," 18 "suspicious," and 27 "negative."

[1] We have calculated our false positives as a percentage of all positive reports. Most authors give them as a percentage of all cases without carcinoma, which shows them in a much more favourable light.

There were three false-positive and seven false-suspicious reports. Macdonald *et al.* (1963) correctly diagnosed 94% of the 72 malignant tumours involving the oesophagus in their series of 179 patients and gave no false-positive reports. Using swabs taken from suspicious areas, Messelt (1960) has obtained 90% positive and 5.7% suspicious cytological findings among 141 malignant tumours of the oesophagus, with only one false positive.

If results like the last two mentioned could be obtained consistently, this would be regarded as the most reliable of all applications of cytodiagnosis. But attention has been drawn to confusing appearances seen in ulcerative oesophagitis (Koss and Durfee, 1961), and the difficulties are certainly worst in exactly those cases where the diagnosis is in doubt on other grounds.

Stomach

Much of what has been said above concerning carcinoma of the lung applies also to the stomach. The prospect of obtaining and examining gastric washings from symptom-free persons in order to discover precancerous states and very early carcinoma is so daunting that it does not seem to have been attempted. Even the screening of persons at extra risk—for example, cases of pernicious anaemia or cases shown on preliminary testing to have achlorhydria—is recommended by only a few enthusiasts, and no curable lesions discovered in this way seem to have been reported.

Rubin and his colleagues (MacDonald *et al.*, 1964), with a very large experience of this method, have reported a series of 500 achlorhydric or hypochlorhydric persons, and found three gastric carcinomas, none of which was early or otherwise undiagnosable ; and there was one false positive. The remarkable series of intramucosal carcinomas reported by Schade (1960) were found among patients being investigated for gastric symptoms.

Another difficulty is that the stomach is not easily accessible to directed biopsy. If a positive cytological report is not associated with a radiological lesion the surgeon is unlikely to feel enough confidence in the cytologist to resect the stomach on these grounds alone, and no minor confirmatory procedure is available.

With these reservations, the cytological examination of gastric contents can be considered a useful procedure, giving about the same rate of diagnostic successes and failures as sputum examination for carcinoma of the lung. It entails much more in the way of effort and time, so that it should be undertaken only in centres where the facilities will not be strained to the detriment of more rewarding work.

We have used the technique recommended by Schade (with normal saline as the washing fluid) except that fasting juice has been rejected and we have examined only the washings.

In the period 1957–63 we have examined gastric washings from 293 patients, and the results in 279 cases for which a final diagnosis is available are shown in Table II: 50% of cases of carcinoma of the stomach were cytologically positive, and there was one false-positive report.

Our experience has not been large enough to give us a very high degree of confidence in reporting, especially as a comparison between direct smears and sections has demonstrated a certain " overlap " between the most alarming appearances of benign cells and the most harmless-looking of malignant ones (Richards and Spriggs, 1961). A supplementary method which should not be neglected is the examination of sections of centrifuged sediment (Richardson *et al.*, 1949). This can easily be carried out along with the smear method, and in some cases the sections are easier to interpret.

TABLE II.—*Results of Follow-up in 279 Patients Whose Gastric Washings were Examined for Tumour Cells* (1957–63)

Final Diagnosis	Cytologically Positive or Suspicious	Cytologically Negative	Unsatisfactory
Carcinoma of stomach ..	29	25	4
Carcinoma of lung ..	1		
No carcinoma	1	215	

Colon

In spite of reports of excellent results (tabulated by Oakland, 1961) cytological examination of the colon is still a very uncommon investigation. The reason lies in the careful preparation of the patient that is necessary before satisfactory specimens can be obtained. The presence of more than a trace of faeces makes the examination worthless, so the patient has to be given repeated enemas until the return is clear. Where it is possible for the preparation to be carried out under the control of the cytologist results can be expected which equal in accuracy those from sputum or gastric washings (Raskin and Pleticka, 1964). We have no personal experience of this method.

Urinary Tract

As the microscopy of urinary sediment has been a standard investigation for so long, it might be expected that the cells of the urine would have been thoroughly described and illustrated, like, for example, those of the blood. The fact that this is not so can be attributed to the poor preservation of cells in this medium, and it must be admitted that the identification of many of those seen is a matter of uncertainty.

In many cases of established carcinoma of the bladder it is possible to identify malignant cells in the urine without difficulty. This examination has, however, not proved very useful in hospital practice, because the diagnosis can readily be made by other means. It is of much more importance in industrial medicine. In the dyestuffs industry, where carcinoma of the bladder is an occupational hazard, regular cytological examination of the urine (Crabbe *et al.*, 1956) is clearly a more acceptable test than routine cystoscopy.

In the general population the " pick-up " rate of bladder tumours is extremely low ; in the series of Riaboff (1954) one case of carcinoma of the bladder was found among 1,738 asymptomatic men ; and in Macfarlane's (1963) series of 6,500 hospital patients no case of carcinoma of the bladder was discovered.

False-positive mistakes are of little significance in a screening test if no diagnostic weight is placed on the report when other investigations are negative. Unfortunately, cystitis sometimes causes marked nuclear abnormalities, and most series of cases examined for diagnosis (as opposed to detection) have included rather numerous false-positive reports. In the large series of Foot *et al.* (1958) more than a third of all positive or suspicious reports were false ; if suspicious (class III) reports are ignored the figure is 10% (this includes cases suspected of carcinoma of the kidney and prostate). Umiker (1964) has published a table showing the errors in several series with similar or worse results.

Tumours of the kidney rarely shed cells into the urine. Their identification is usually considered difficult, and in any case they appear only at a late stage.

In our own series smears of urine sediments have been made by two methods in parallel: wet-fixed smears stained by Papanicolaou's method, and air-dried smears stained with May–Grünwald and Giemsa stains. The first of these has been found the most appropriate for this material.

A reasonably certain final diagnosis is available in 415 cases, most of them with symptoms referable to the urinary tract. The results are shown in Table III.

It will be seen that there were three false-positive reports from patients without tumours of the urinary tract. Two of these were in cases suspected to have carcinoma of the kidney, and one of the bladder. A positive report was also given in four cases with a final histological diagnosis of papilloma of the bladder, and it is questionable whether these should be classed as false positives or not. (In a screening programme they would of course be regarded as correctly detected cases.) Most papillomas shed cells which are not clearly abnormal, but a heavy deposit of columnar cells may suggest the correct diagnosis.

TABLE III.—*Results of Follow-up in 415 Cases Whose Urine was Examined for Tumour Cells* (1953–62)

Final Diagnosis	Cytologically Positive or Suspicious	Cytologically Negative for Carcinoma
Carcinoma of bladder { Primary	74	51
Carcinoma of bladder { Secondary	4	9
Papilloma of bladder	4	24*
Carcinoma of kidney	3	14
Carcinoma of prostate	1	4
No tumour	3	224

* In four of these the cytological picture was reported as that of papilloma.

We have no experience of cytological diagnosis from prostatic smears. It appears that the "occult" symptomless prostatic carcinomas which are common in old men do not usually shed recognizable cells, and that a finding of malignant cells nearly always signifies clinically active carcinoma. Although the method has been considered highly reliable (Fergusson and Gibson, 1956), it is difficult to confirm the findings in an ordinary series, since operation is often not contemplated and survival may be long (Bamforth, 1958). In a condition where it is often impossible to find out whether one's opinion has been right or wrong the whole foundation of accurate reporting is missing.

Serous Fluids

In most diagnostic applications of cytology our results have been much the same as those of other workers in the same field. In the case of pleural and peritoneal fluids this is not so. From the beginning we have used the methods of haematology in preference to the Papanicolaou technique, and our results are very much better than those usually recorded from the cell-block method or the Dudgeon or Papanicolaou or acridine-orange techniques. In particular, we have been able to avoid false-positive errors almost completely. This cannot simply be due to long experience, because in the seven years before 1957 not a single false-suspicious or false-positive report was given, and the few mistakes which have been made occurred in subsequent years when our experience was greater.

The technique used is described by Spriggs (1957b), and is virtually the same as that recommended by Balduini (1947): a very concentrated deposit of cells is spread on a slide so as to dry instantaneously, and is then fixed in methyl alcohol and stained by the May–Grünwald and Giemsa technique. Papanicolaou smears are also made for comparison; Naylor and Schmidt (1964) have achieved a good accuracy with this method, but we do not find any advantage in it. Sections of fixed sediment are also useful if large cell clumps are present.

Table IV shows our results from 1953 to 1962. A diagnosis of malignant effusion has been accepted as correct if it was consistent with the clinical course and the patient died of the disease, even if no post-mortem examination was performed.

The percentage of cases in which malignant cells are found differs according to the primary growth; for instance, 92% in malignant ascites due to carcinoma of the ovary, 72% in pleural effusion due to carcinoma of the breast, and 54% in pleural effusion due to carcinoma of the lung.

Because of the reliability of this examination we generally express reports in unequivocal terms—for example, "numerous malignant cells present, oat-cell type." In a few cases a lesser degree of confidence was felt, and the report read "a few cells very suspicious of carcinoma" and the like.

TABLE IV.—*Results of Follow-up in 1,986 Cases of Pleural and Peritoneal Effusion* (1953–62)

Final Diagnosis	Cytologically Positive or Suspicious*	Cytologically Negative
Malignant disease { Pleural	319	205
Malignant disease { Peritoneal	160	64
No malignant disease { Pleural	4	1,056
No malignant disease { Peritoneal	0	178

* 440 were reported unequivocally "positive" and only 43 "suspicious." There were one false-suspicious and three false-positive reports.

Pleural or peritoneal effusions complicating malignant disease do not necessarily result directly from seeding of metastases in the serous membrane. When no malignant cells are found this is probably in most cases because they are not present in the slides examined, but in an unknown proportion they are present but not recognized. Certain histochemical methods have been tried in the hope of distinguishing tumour cells from aberrant mesothelial cells, but the only one which we have sometimes found useful is the periodic-acid–Schiff. Mesothelial cells, as well as some tumour cells, have positive granules or diffuse staining, but a spherical positively stained body at the cell centre, and often distorting the nucleus, is diagnostic of a mucous vacuole. Mucus-secreting carcinoma cells are the only type in which such vacuoles have been seen (Spriggs, 1957b). The material is not removed by salivary digestion, but we have sometimes found it difficult to achieve an all-or-none distinction between glycogen and mucin by this procedure.

Serous fluids are very little trouble to examine. Whereas a single smear of sputum may take 10 minutes to scan, a glance at a pleural-fluid slide will often reveal malignant cells. With our technique the cells are close together and thousands are seen in one low-power field. Each smear occupies only a fraction of the slide and seldom takes more than two minutes to scan. Malignant cells when present are generally visible in every smear, so that it is unnecessary to examine more than three or four.

It is sometimes possible to give an opinion about the probable site of the primary growth. This applies particularly to the lung, as the so-called "oat cells" are very characteristic and are mimicked by only a few relatively rare tumours—for example, sympathicoblastoma and small-celled sarcomas. Mucus secretion and acinar formations are evidence of adenocarcinoma, but give no information about the site unless it is already suspected. Melanomas occasionally produce melanin-containing tumour cells in effusions. Squamous carcinomas are very rarely identifiable from evidence of cornification, because the more differentiated types do not seem to produce pleural seedlings.

The cytological examination of serous fluids is useful in diagnosis whether malignant disease is in question or not, but of course it has no relevance to the early or curable cancer case. Its main value is that a positive report will sometimes prevent the necessity for other procedures.

Breast

The cytological examination of discharges from the nipple has been successfully used in a few centres (Papanicolaou *et al.*, 1958; Marsan and Bertini, 1960) but we have had no experience of it. Breast tumours are rather inaccessible and this seems a promising approach, but discharges are found in only a minority of cases. Samples of fluid aspirated from cysts of

the breast have been received from time to time in our laboratory; out of the series of 67 cases cells suspected of malignancy were found in only one case. Two cysts on opposite sides were examined at 21 months' interval, and both were correctly reported "positive." There was one false negative in the series, the smear showing blood only; and there were no false positives.

Cerebrospinal Fluid

In cases where the meninges are infiltrated by malignant cells, these can be found in the cerebrospinal fluid.

Excluding leukaemia, we have encountered malignant cells in the cerebrospinal fluid in 59 cases during the past 12 years. These were made up as follows: 21 carcinomas (10 lung, 5 breast, 2 cervix uteri, 2 ethmoid, 1 each of colon and skin), 3 sarcomas, 1 melanoma, 1 plasmacytoma, 25 gliomas (including 7 medulloblastomas), and 8 in which the primary growth is unknown.

Difficulties of identification are not very great, but if the cell-count is low it may be difficult to make good smears. May–Grünwald and Giemsa staining has been used, with a simple method described by Spriggs and Boddington (1959). Good results can also be obtained even with very small samples, using the apparatus of Sayk (Sayk, 1960; Bots *et al.*, 1964). We do not recommend wet fixed films stained by the Papanicolaou method, but they can be used with success as shown by Naylor (1964). The presence or absence of malignant cells is not the only information required from stained films of cerebrospinal fluid, and as the quantity of material is limited we make air-dried films only.

Malignant Cells in Circulating Blood

Much of the literature of this subject can be discounted, either because the techniques were inappropriate for identifying the cells of white-cell concentrates or because the great variety of "rare" cells was not realized and proper control observations were not made. The pendulum has now perhaps swung back too far, and doubts are expressed whether malignant cells are identifiable at all in this material.

With first-rate technique it is possible to examine the cells of white-cell concentrates in excellent detail (see the photographs of Stofberg, 1963), and there is no doubt that a few patients with metastasizing tumours do have circulating malignant cells, sometimes in considerable numbers. As this phenomenon is infrequent (perhaps 5% of patients with advanced carcinoma) an examination of the blood for malignant cells is of no clinical importance.

Miscellaneous

From 1952 to 1963 there were 501 specimens submitted for examination for malignant cells which do not fit into any of the categories dealt with already. Many of these were aspirates from cysts. It is worth mentioning that cyst fluids are isolated from the normal scavenging mechanisms, and therefore contain debris, dead cells, and sometimes cholesterol crystals. This is very different from the usual contents of the pleural and peritoneal cavities, consisting almost entirely of living cells. It is therefore sometimes possible to state that a sample supposedly of peritoneal fluid is in reality from a cyst—for example, of the ovary.

The large subject of tissue aspirates (tumours, lymph nodes, bone marrow, spleen, etc.) will not be dealt with, as they are not routinely examined in this laboratory.

Summary and Conclusion

The results of our experience over 12 years, together with other published figures, are presented as a guide to the most important applications of cytodiagnosis.

This type of examination is useful in two different ways: as a screening test for the detection of symptomless disease, particularly precancerous states; and as a diagnostic aid when biopsy is inapplicable.

As a screening test the cervical smear has been outstandingly successful and carcinoma-in-situ can now be found and treated in whole populations, so that cervical carcinoma has become a preventable disease. Cytological examination of the urine is of established value in industrial preventive medicine. Other applications to cancer detection are at present of dubious or minor importance.

As a diagnostic procedure, cytological examination is applicable to sputum, washings from the gastro-intestinal tract, urine, serous fluids, cerebrospinal fluid, and various other materials. With experience a good standard of accuracy can be attained, but this varies with the type of material as well as with the observer. Avoidance of "false-positive" reports is of the greatest importance in diagnostic work, whereas in screening the emphasis is on sensitivity (avoidance of "false negatives").

In research applications a substantial false-positive rate disqualifies the whole of any series in which it occurs.

It is very important to realize that no person, however expert in identifying malignant cells in any particular material, is in a position to pronounce about the cells in specimens with which he is unfamiliar. He must first study a series of relevant samples for which the answers are known, and refrain from making reports until he finds that his opinions are correct. The identification of tumour cells has to be learnt anew in each different context, and the errors of the observer and of the technique allowed for, before any results are acceptable.

With this proviso, cytological examination can be considered a useful, and in some situations an indispensable, method of diagnosis of cancer; moreover, it holds the unique promise, by population-screening, of eliminating at least one common type of malignant disease from the community.

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6-Phosphogluconate Dehydrogenase Activity in Vaginal Fluid: Limitations as a Screening Test for Genital Cancer

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Recognition that the commoner forms of cancer of the genital tract in women can be cured if treated at an early stage has focused interest in screening tests which can be applied on a wide scale. The current practice of examining cells in smears obtained from the vagina and uterus for evidence of cancerous change is of proved value (MacGregor and Baird, 1963) but some simpler alternative would be welcome. Bonham and Gibbs (1962) and Bonham (1963) have reported encouraging results, using 6-phosphogluconate dehydrogenase (6-PGD) activity in vaginal fluid as a screening test for uterine cancer. We have independently carried out this biochemical assay in parallel with standard cytological and histological techniques and this report summarizes our findings in certain well-defined clinical groups.

Material and Methods

Most of 2,480 patients examined were attending gynaecological out-patient departments in London hospitals. Antenatal and post-natal patients were included. Some women were in-patients at the Royal Marsden Hospital with suspected uterine cancer.

Samples of vaginal fluid were collected and 6-PGD activity was determined, using the method of Bonham and Gibbs (1962) modified for a Technicon AutoAnalyzer (Weg and Cameron, to be published). Following Bonham (1963), we accepted 80 units of 6-PGD per g. of dry weight[1] of sample as the upper limit of normal. Values above this figure were called positive results.

Smears were taken for cytology from the same patients at the same time and reported on by one of four pathologists, using prearranged standardized criteria. Where malignancy was suspected on cytological grounds the final diagnosis was based on histological examination of biopsy material.

Results

Women with Genital Tract Cancer.—Sixty-six women were found on histological grounds to have some malignant lesion of the genital tract, and Table I summarizes our results in these patients. A positive result was obtained in 53 out of 55 patients with invasive uterine cancer. The result was negative in two patients (both with adenocarcinoma corpus) who had undergone thorough curettage two weeks previously and in whom cytology showed no malignant cells.

TABLE I.—*Results of 6-PGD Assay in Patients With Uterine Carcinoma*

	Positive	Negative	Total
Carcinoma-in-situ	6	5	11
Epidermoid carcinoma	36	0	36
Adenocarcinoma cervix	4	0	4
„ corpus	9	2	11
Other pelvic carcinoma secondarily invading genital tract	4	0	4

Women Without Genital Tract Cancer.—A high incidence of positive enzyme results was found in women with no clinical or cytological evidence of malignant disease of the genital tract. The percentages of false-positive results found in different age groups are shown in Table II. Before the menopause about one woman in three shows increased enzyme activity, and this ratio increases markedly after the menopause. The phase of the menstrual cycle in which the

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[1] The dry weight is not a convenient parameter as it entails freeze-drying each sample and weighing containers with and without sample. It is also inaccurate when samples are small—that is, less than 3 mg. dry weight. We have estimated soluble-protein concentrations in more than 1,000 samples and found that 6-PGD activity per mg. soluble protein in general parallels the enzyme activity per mg. dry weight of sample. For direct comparison with other reported work, however, the dry weight parameter has been used in this paper.