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THE CYTOLOGICAL DIAGNOSIS
OF SOLID TUMOURS BY SMALL
NEEDLE ASPIRATION AND ITS
INFLUENCE ON CANCER
CLINIC PRACTICE*

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FOR SOME 20 years the Ontario Cancer Foundation's London Clinic, in conjunction with the Department of Pathology of the University of Western Ontario, has been studying the value of tumour aspirates as interpreted histologically from cut sections. Out of sheer curiosity a supplementary smear from the same material was occasionally made; indeed, at times from necessity when tissue fluid alone was obtained, or when the tumour proved to be cystic, the smear technique was used by itself. Gradually, and in particular since 1954, our curiosity has been replaced by the realization that the cytological interpretation of tumour aspirates has established itself as a most valuable procedure — surpassing and replacing the study of the sectioned aspirate in some instances and equalling it in others. But in our hands it is as yet inferior for certain sites, for example, in the diagnosis of primary malignant tumours of lymph nodes. Reliance is now placed on cytology to the extent that, when an aspiration is performed on an accessible lump, emphasis is placed first on obtaining tissue fluid for cytology, the plug when and if procured forming a very important but secondary component of the procedure.

The authors feel it superfluous to review this subject from its historical and bibliographical aspects. For an excellent coverage of the literature and original contributions, the superb articles of Martin and Ellis (1930),¹ Stewart (1933),² Godwin (1956)³ and Papanicolaou (1958)⁴ are both timely and complete. This presentation is not concerned

with exfoliative cytology or the cytology of effusions.

The successful interpretation and clinical application of the cytology of solid tumours depends, in a large measure, on four principal factors, without which the patient could be the loser:

1. The experience of the pathologist and clinician.
2. The attitude of acquiring perfection.
3. The challenge of participating in and exploring the cytological diagnostic field to its limit.
4. The clinician's ability and willingness to accept the responsibility for positive, negative, and equivocal reports, in particular the latter two.

TECHNIQUE

The aspiration set consists of a 50 c.c. Luer-Lok syringe, 20- and 22-gauge needles of various lengths, and freshly prepared slides coated with egg albumin. Actually, a wide assortment of sterile needles varying up to 13-gauge is readily accessible.

The overlying skin is sterilized. If the No. 22 needle is routinely used, no local anæsthetic is required. The tumour is immobilized with the left hand, and the needle is inserted until the tumour or its capsule is engaged. Suction to a maximum is now employed, and the needle is guided through the tumour in two or three directions. Before withdrawing the needle all suction is released, thereby preventing aspiration into the lumen of the syringe. When withdrawn, the tissue fluid within the needle is expressed on to the albuminized slides, two preparations being made. The material is smeared evenly by using the needle and, while the smear is still moist, the slides are immersed in fixative in a Coplin jar. Should a plug or excess tissue be available, it is placed in fixative for histological section. Gentle pressure is applied over the aspirated site to minimize the possibility of extravasation.

PATHOLOGICAL ASPECTS

The smear is fixed for 15 minutes to one hour in fluid containing 1% glacial acetic acid in 95% ethyl alcohol. After fixation, and while still wet, it is stained with hæmatoxylin and eosin in the usual way; it is then mounted and ready for microscopic examination.

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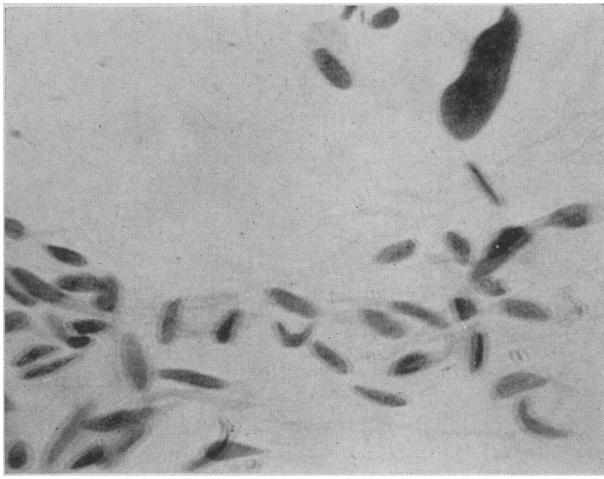


Fig. 1.—Smear of aspirate from pre-auricular lymph node, metastatic melanoma. Large spindle-shaped cells with anaplastic nuclei. Large cell with giant nucleus in right upper corner.

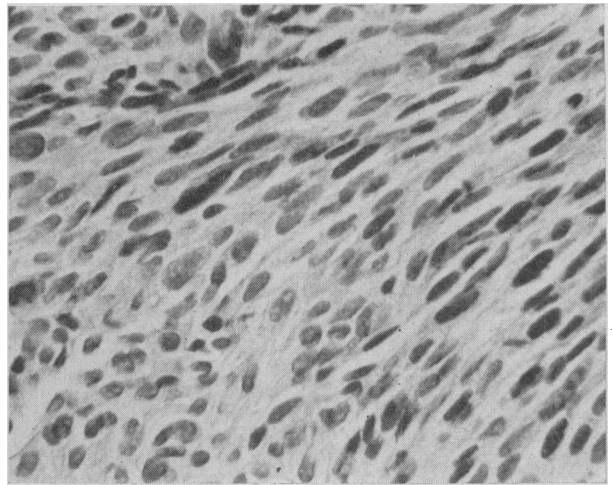


Fig. 2.—Same case as Fig. 1. Paraffin section of plug of tissue obtained by aspiration biopsy. Anaplastic spindle-shaped melanoma cells.

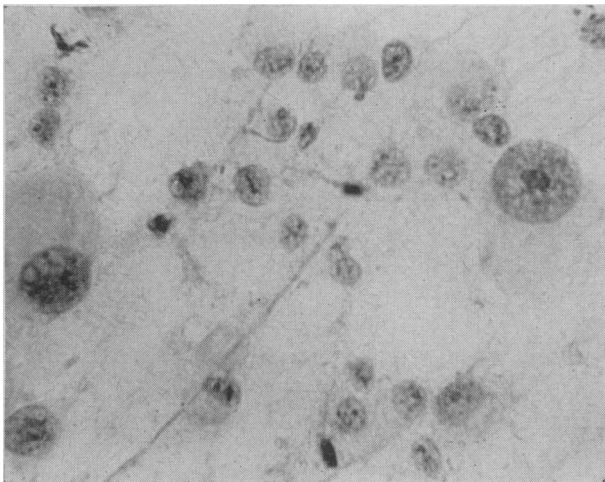


Fig. 3.—Smear of aspirate from cutaneous nodule of metastatic melanoma. Two cells with very large nuclei. Note macronucleolus.

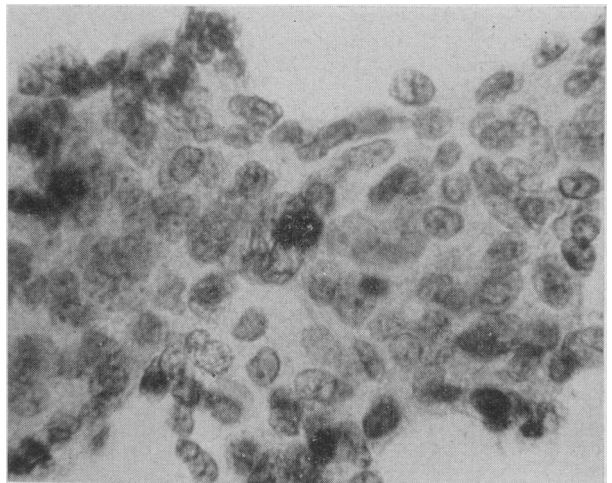


Fig. 4.—Smear of aspirate from breast. Aggregate of malignant polyhedral epithelial cells.

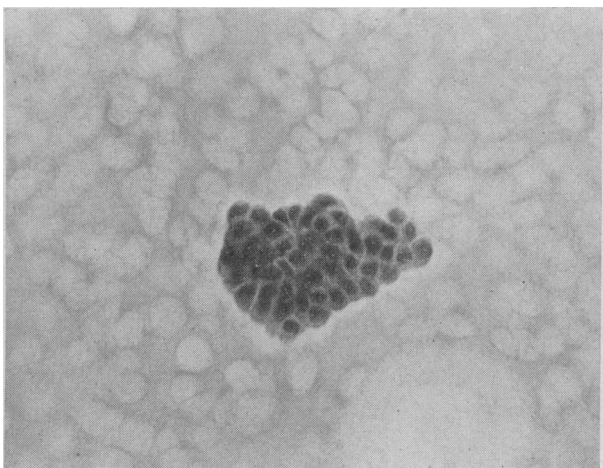


Fig. 5.—Smear of aspirate from breast. Aggregate of well-differentiated malignant epithelial cells.

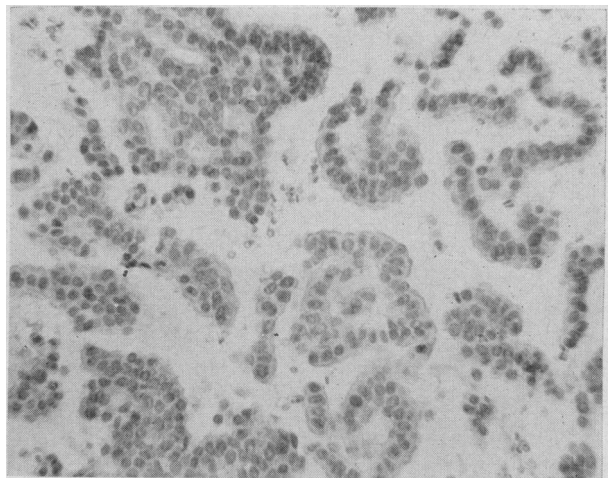


Fig. 6.—Same case as Fig. 5. Paraffin sections of tissue aspirated from axillary lymph node. Well-differentiated malignant glandular epithelium assuming a glandular pattern.

NOTE: (1) All smears and paraffin sections were stained with hæmatoxylin and eosin. (2) All photomicrographs are $\times 460$, except Fig. 6, which is $\times 230$.

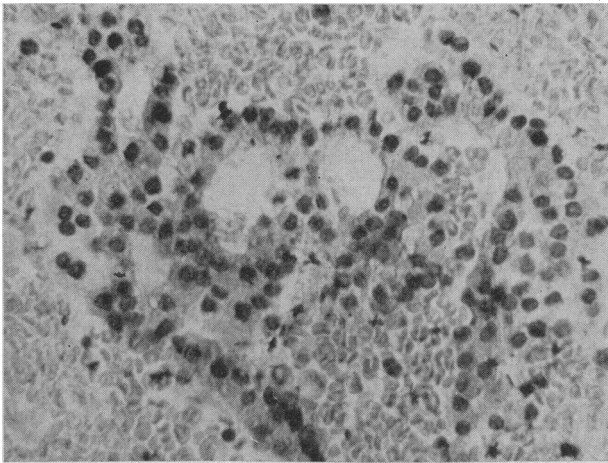


Fig. 7.—Paraffin section of coagulum aspirated from iliac bone, metastatic carcinoma of kidney. Malignant glandular epithelium forming tubular structures.

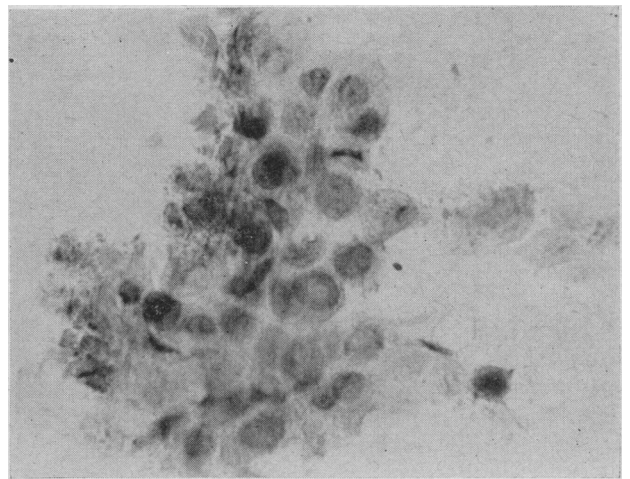


Fig. 8.—Smear of aspirate from lymph node of lower back, metastatic squamous cell bronchogenic carcinoma. Aggregate of anaplastic polyhedral epithelial cells.

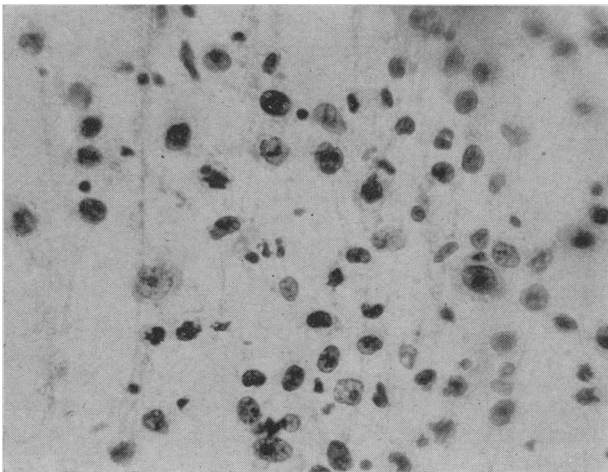


Fig. 9.—Smear of aspirate from cervical lymph node containing lymphocytes and atypical reticulum cells, reticulum cell sarcoma. Reticulum cells are the large pale cells with a vesicular nucleus and a prominent nucleolus.

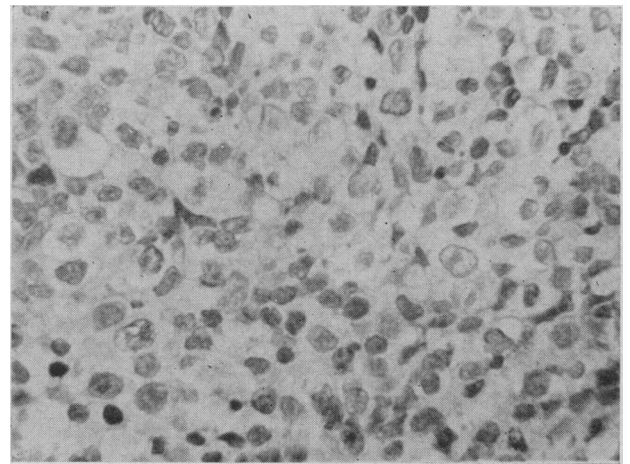


Fig. 10.—Paraffin section of tissue aspirated from cervical lymph node, reticulum cell sarcoma. Sheets of angular cells with a large pale, sometimes indented nucleus, and prominent nucleolus.

Interpretation and comments.—If smears are prepared well, properly fixed and stained well, the cytological detail is excellent. In our experience better cytological detail is obtained when smears are fixed while still moist, and stained while still wet after fixation, as in the Papanicolaou technique. If it is not possible to stain immediately after fixation, the smear is flooded with glycerin and covered with a glass slide. This delayed technique gives reasonably satisfactory results but immediate staining is preferable. If the smears are air-dried before fixation, the cells appear somewhat shrunken, the nuclei being smaller and more intensely stained. If the aspirate contains much blood or other protein-rich coagulable fluid, smears are generally unsatisfactory. Under such circumstances the neoplastic cells are embedded in an intensely eosinophilic matrix, and the cytological detail tends to be smudgy and blurred. While Papanicolaou's alcohol-ether fixative is an excellent one, ether has been eliminated from the fixative which we use because

it is so volatile. One per cent concentration of glacial acetic acid in 95% ethyl alcohol has been employed because it is a good protein precipitant; it intensifies nuclear staining and has proven satisfactory in our experience.

The pathologist must take a keen interest in this method of diagnosis. He must be willing to learn a type of tissue diagnosis different from the conventional section of embedded tissue. He must become conscious of the importance of fine cytological detail of individual cells. In smear preparations, tissue infiltration and invasiveness, cell arrangement and pattern of growth are lacking. A diagnosis of malignancy must be made on such cytological details of individual cells as nuclear anaplasia, nuclear-cytoplasmic ratio, presence of a macronucleolus, bizarre cells and abnormal mitoses. It is possible to make an accurate diagnosis on exceedingly few cells. This method is time-saving and can be repeated with safety. If necessary, for example, with a patient waiting in the clinic for a diagnosis before starting treatment,

TABLE I.—ASPIRATION SMEAR CYTOLOGY, APRIL 1955 - APRIL 1958

	Total cases reviewed	Total cytological preparations
Malignant.....	203	291
Benign.....	194	233
Total.....	397	524

The Ontario Cancer Foundation London Clinic, London, Canada.

a report can be given in as short a time as 30 minutes after aspiration of the lump. Analysis of results, presented elsewhere in this paper, indicates that the method, subject to critical appraisal, is satisfactorily reliable.

As a rule, a diagnosis of metastatic squamous cell carcinoma is established readily in smear preparations. The presence of fragments of keratin, keratinizing atypical squamous epithelium and aggregates of cells showing undoubted malignant characteristics is justification for a diagnosis of

employed in 203 cases of proven cancer, and in 194 cases of benign disease. Table I indicates a total number of 524 cytological preparations.

A breakdown of the benign group is presented in Table II, and it is apparent that lesions of the breast and lymph nodes comprise the bulk of our benign material.

Reference to Table III reveals again that lesions of the breast and lymph nodes were major problems in this malignant series. From 100 cases of primary or recurrent breast cancer, 150 cytological preparations were made, 109 of which were positive or probably positive for cancer cells. In 90 cases, both of primary and metastatic lesions of lymph nodes, 118 smears were studied of which 90 were positive or probably positive. "Probably positive" was not regarded as proof positive of the presence of cancer, and aspiration was generally repeated. From the last column it is noted that of 291 cytological preparations in proven cancer, 216 (75%) confirmed the diagnosis and 75 (25%) were negative. We do not regard these 75 smears

TABLE II.—ASPIRATION SMEAR CYTOLOGY, BENIGN CONDITIONS

	Breast benign cystic disease	Breast fibroadenoma	Breast inflammatory disease	Lymph-adenitis	Parotid (benign)	Thyroid disease	Miscellaneous benign conditions	Total
Number of cases examined.....	91	17	14	34	8	13	17	194
Number of cytological preparations examined	109	19	17	39	9	17	23	233

The Ontario Cancer Foundation London Clinic, London, Canada.

malignancy. The smears of aspirates of carcinoma of the breast usually contain many individual cells and aggregates of cells showing readily recognizable characteristics of malignant cells. Smears of some benign lesions of the breast, such as mammary dysplasia, occasionally fibroadenoma, and infrequently duct papilloma, contain surprising amounts of epithelium. The firm cohesion of the cells, sometimes in little strips like a lining, and their uniform, frankly innocent appearance usually indicate their benign nature.

ANALYSIS OF DATA

In this particular study period from April 1955 to April 1958, aspiration smear cytology was

as true false-negatives, since a repeat smear confirmed the diagnosis of malignancy in most instances.

Experience with carcinoma of the breast is summarized in Table IV. In 92 of the 100 cases of both primary and recurrent cancer of breast, confirmation of the histological diagnosis was obtained by the cytological method. It is also revealed that in 16 cases, or 18%, more than one aspiration was required to establish a confirmatory diagnosis. In comparison with published data for the same site, using histological data in addition,³ this percentage of negative smears could be anticipated, in that material was aspirated by operators varying in experience. It is our impression that accuracy of diagnosis increases as the operator gains expe-

TABLE III.—ASPIRATION SMEAR CYTOLOGY, MALIGNANT DISEASE

Cytologic diagnosis	Breast, primary (80 cases) No. smears	Breast, recurrent (20 cases) No. smears	Lymph node primary (20 cases) No. smears	Lymph node metastatic Ca. (70 cases) No. smears	Malignant melanoma (3 cases) No. smears	Miscellaneous malig. diseases (10 cases) No. smears	Total
"Positive for cancer cells".....	73	17	18	65	12	3	188
"Probably positive for cancer cells".....	15	4	0	7	0	2	28
"No cancer cells".....	31	10	8	20	0	6	75*
Total.....	119	31	26	92	12	11	291

*Seventy-five first smears (25% of total) did not support a diagnosis of cancer and were repeated. The Ontario Cancer Foundation London Clinic, London, Canada.

TABLE IV.—ASPIRATION SMEAR CYTOLOGY, CARCINOMA OF THE BREAST

	Examined		Diagnosis confirmed by cytology		False negative	Diagnosis of malignancy established on first aspiration		More than one aspiration required for diagnosis
	Cases	% of total examined	Cases	% of total examined		Cases	Cases	
Primary cases.....	80	100%	74	93%	6	61	82%	13
Recurrent cases.....	20	100%	18	90%	2	15	83%	3
Total.....	100	100%	92	92%	8	76	82%	16

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rience with the technique. In the entire series of 100 cases of histologically proven cancer of breast, there were eight in which all smears were negative. These were regarded as true false-negative aspirations and will be dealt with subsequently.

Table V is based on 70 cases of metastatic carcinoma involving lymph nodes and 20 cases of primary malignant lymph node disease. Although reference has been made to the difficulties of establishing a diagnosis in the latter group, it is surprising how often one can cytologically distinguish malignant from benign conditions. To the clinician it is of real importance to establish the presence or absence of significant malignant lymphadenopathy in a site distant to that on which a histological diagnosis has already been made. Sixty-six of 70 cases of metastatic cancer in lymph nodes were confirmed by cytology, only four of which required repeat aspiration.

As a final appraisal, reference must be made to both false-negative and false-positive smears. A total of 18 in 203 malignant cases were regarded as true false-negatives. The breast group accounted for 8 of the 18. We would attribute most of these errors to the small size of lesion and perhaps a geographical miss, or inability of the 22-gauge needle to penetrate a very scirrhous tumour. Regardless of the accuracy and carefulness of both operator and pathologist, false-negative results are bound to occur, as with biopsy in a small proportion of cases.

Similarly, the occasional false-positive will be reported. In this entire series there were only five: two in subsequently proven cases of fibroadenomata of breast (one patient was pregnant); one in a

case of microcystic disease of breast; one in a benign lymphadenopathy; one, the final and most interesting case, was one in which an organizing bursitis was interpreted as a chondrosarcoma. But again it should be repeated that, with careful appraisal, a false-positive report should prove to be of little clinical consequence.

THE INFLUENCE OF CYTOLOGY ASPIRATION ON CANCER CLINIC PRACTICE

There are sufficient numbers of inflammatory swellings referred to a cancer clinic as possible new growths that one is compensated in dealing with these by quickly establishing the diagnosis and passing on information as to bacterial type and sensitivity. Without enumerating, one thinks of the occasional tuberculous adenitis, actinomycosis, or non-specific suppurative adenitis uncovered. At a stage before clinical suppuration the inflammatory exudate, as revealed in the smear, is the diagnostic clue.

In the parotid, thyroid and miscellaneous groups listed in Table II, insufficient experience has been gained from the limited volume for a sensible appraisal, but immeasurable assistance is often obtained in such isolated lesions as the bone tumour, soft tissue tumour, accessible cyst, salivary tumour, and many others. The aspiration of Virchow's node occasionally gives a lead in the diagnosis of some deep-seated, yet disseminated, malignancy; or, if positive, it alters the management of entities such as mammary or bronchogenic cancer. Many such individual experiences serve in lending support to the enthusiastic impression of the importance of cytology that we have gained

TABLE V.—ASPIRATION SMEAR CYTOLOGY, MALIGNANT LYMPHADENOPATHY, PRIMARY AND SECONDARY (METASTATIC CARCINOMA)

	Examined		Diagnosis confirmed by cytology ("malignant cells")		False negative	Diagnosis of malignancy established on first aspiration		More than one aspiration required for diagnosis
	Cases	% of total examined	Cases	% of total examined		Cases	Cases	
Primary malignant lymphadenopathy	20	100%	17	85%	3	15	88%	2
Metastatic carcinoma in lymph nodes	70	100%	66	94%	4	62	94%	4
Total.....	90	100%	83	92%	7	77	93%	6

The Ontario Cancer Foundation London Clinic, London, Canada.

from a study of the major and more common groups encountered.

Breast.—Tumours of breast, being so common and so accessible, are particularly well suited for aspiration cytology. From Table IV it is seen that of 80 primary cases of breast cancer 74, or 93%, were diagnosed cytologically. Although not eliminated, the frozen section need seldom be used. In a clinical research series, our radiotherapy staff has now extended the use of preoperative cobalt-60 therapy to include stage 1 breast cancer: previously, such management was recommended for the obviously clinical stage 2 group. So little has been done differently for early breast cancer in the past 30 years that it becomes a little painful psychologically to continue to pin one's faith on the established routine of frozen section, surgery and postoperative irradiation. Establishing proof of the early lesion permits a clinical research approach in the way of preoperative tumour devitalization by radiomimetics or ionizing rays.

Perhaps the most dramatic effect of aspiration on the patient is proving a questionable breast lump to be cystic. The patient's tense anxiety is relieved. Taking an immediate frozen section is obviated, and a planned approach to the management of this knotty problem is permitted. Of 91 cysts of the breast aspirated, only one was reported as "probably positive", and clinical follow-up of this case proved the cytological finding to be the only false-positive in the series. All others were negative.

A smaller group of inflammatory breast lesions appear in most cancer clinics. The exudative type of cytology is readily recognized and appropriate treatment arranged.

Metastatic cancer in lymph nodes.—Table V shows that of 70 such cases, 94% were confirmed by cytology. The significance of such accuracy is applicable in particular to nodes secondarily involved from cancer of the head and neck. One learns to detect clinically the malignantly involved node or the node within normal limits. At times, the clinical impression can be wrong for either category. It is not suggested that aspiration be done in all circumstances, but when in doubt with the "questionably significant" node it is safer to establish diagnosis immediately than "review in one month". Earlier diagnosis, therefore, with all it implies, is the main point gained by this procedure.

There is an older age group in which it is important to establish a diagnosis for two main reasons: if primary oral cancer, for example, is being treated by external irradiation one would include an adjacent (cytologically positive) node in the treatment field; and secondly, if the patient's general condition precludes extensive surgery to remove a lymph node known to be invaded, it is a much simpler problem to manage curatively by irradiation when tiny than if grossly malignant.

Primary lymph node tumours.—Smear diagnosis by aspiration of primary malignant lymphomas has

not been generally reliable in our hands. Occasionally it has been possible to make a reasonably certain diagnosis of Hodgkin's disease or reticulum cell sarcoma by such a method, but the diagnosis of lymphosarcoma and lymphatic leukæmia has been virtually impossible by the smear method. Features such as lymph node architecture, invasion of capsule and perinodal tissue, and appearance of follicles cannot be discerned by smear. The study of the whole lymph node is so often required for a correct diagnosis that for the time being we are reluctant to concede that cytological examination is an acceptable diagnostic substitute.

SUMMARY

An appraisal has been made of the accuracy and applied clinical value of aspiration cytology in 203 cases of cancer and 194 cases of deep-seated benign disease. A total of 524 smears was made. Exfoliative cytology and the cytology of effusions are not included in this study.

Without anaesthesia, a 22-gauge needle withdraws tissue fluid which is smeared on egg albuminized slides, fixed while still wet and stained by hæmatoxylin and eosin.

The pathologist's conventional attitude based on histology must give way to a cytological approach if the method is to have significant value.

In breast cancer 93% were confirmed cytologically, although in 18% more than one aspiration was required. Establishing diagnosis previously by this method permits a planned therapeutic approach and eliminates the need for frozen section examination in most instances.

In lymph nodes secondarily involved by malignant disease, 94% of diagnoses were confirmed by aspiration cytology, thereby permitting an earlier approach to management.

Although smear diagnosis of primary lymph node cancer is possible in this group, it is still advisable to make the diagnosis histologically, except in unusual cases.

No attempt in this study has been made to evaluate the accuracy of cytological smear examinations in benign breast disease. The psychological effect of immediately establishing the diagnosis of cystic disease, however, is obviously most rewarding.

There were five false-positives (2.5%) in 194 benign cases; if interpreted in the light of clinical impression, this need be of little consequence.

Of 203 malignant cases, 18 (or 8.8%) gave a false-negative result. Eight of the 18 were in breast lesions and required further histological investigation.

The simplicity, accuracy and practical value of aspiration smear cytology for the solid tumour are established, and deserve recognition and wider application.

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RÉSUMÉ

Afin d'obtenir une évaluation du degré de précision ainsi que de la portée clinique de la cytologie d'aspiration les auteurs ont examiné 524 frottis obtenus par cette méthode et recueillis dans 203 cas de cancer et 194 de lésion

bénigne profonde. La cytologie exfoliatrice et celle des épanchements ne furent pas incluses dans cette série. Sans anesthésie et au moyen d'une aiguille de calibre 22 on retire par ponction une gouttelette de suspension tissulaire que l'on étale ensuite sur une lamelle enduite au préalable de blanc d'œuf. Le tout est fixé à l'état frais et teint à l'hématoxyline-éosine. S'il veut conférer quelque importance à cette méthode, le pathologiste devra se départir de son attitude conventionnelle fondée sur l'histologie et en adopter une autre à base de cytologie.

Dans les cas de cancer du sein 93% reçurent une confirmation cytologique bien que dans 18%, plus d'une aspiration fut nécessaire. L'établissement d'un diagnostic par cette méthode permet un acte thérapeutique chirurgical plus rationnel et dans la plupart des cas rend inutile l'examen préopératoire extemporané. Le diagnostic d'envahissement des ganglions lymphatiques fut confirmé dans 94% des cas par cette méthode permettant ainsi d'orienter d'une façon plus précoce la conduite du traite-

ment. On peut toujours obtenir un adénogramme par examen des frottis dans les cas de cancer primaire des ganglions lymphatiques, mais à moins de circonstances particulières, l'examen histologique semble préférable.

Les auteurs n'ont pas cherché dans cette étude à déterminer la précision de l'examen cytologique dans le diagnostic des affections bénignes du sein. L'effet psychologique dans la détermination immédiate du diagnostic de kyste est cependant des plus encourageants. Il y eu cinq résultats faussement positifs (2.5%) dans les 194 cas bénins, erreur qui à la lueur des impressions cliniques est de peu d'importance. Des 203 néoplasmes malins, 18 ou 8.8% donnèrent un résultat faussement négatif. Huit d'entre eux étaient des lésions du sein et exigèrent un examen histologique plus poussé. La simplicité, la précision et la valeur pratique des examens de frottis cytologiques d'aspiration dans les cas de tumeur solide sont établies et méritent une reconnaissance plus étendue et des applications plus nombreuses.

EXPERIENCE WITH SERUM GLUTAMIC OXALOACETIC TRANSAMINASE*

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THE SERUM transaminase (SGO-T) estimation was introduced into clinical medicine by LaDue¹ and co-workers less than five years ago and has become established as a useful diagnostic test in various clinical states. The historical and biochemical background and the clinical application of the test have been reviewed by Conrad² and Wroblewski³ in excellent key articles.

Glutamic oxaloacetic transaminase is present in all cells and is released into the serum with cellular breakdown. Normal human serum contains 4-40 units.³ With injury to cells rich in the enzyme, much higher serum levels occur. The SGO-T test has proved of greatest value as a sensitive indicator of tissue injury when clinical evidence of this injury is uncertain and other laboratory tests give normal or equivocal results.

The purpose of this paper is to report some aspects of our experience with the test that point up its practical diagnostic value. This experience is derived from over 1000 SGO-T estimations on 375 patients studied at the Ottawa General Hospital. The method of Karmen⁴ was used and nearly all the estimations were performed by one technician. Twenty-one estimations on 16 young normal adults gave values ranging from 10 to 40 units.

Heart muscle is particularly rich in GO-T. Some of the highest serum levels are obtained in diseases with injury to the myocardium. Fig. 1

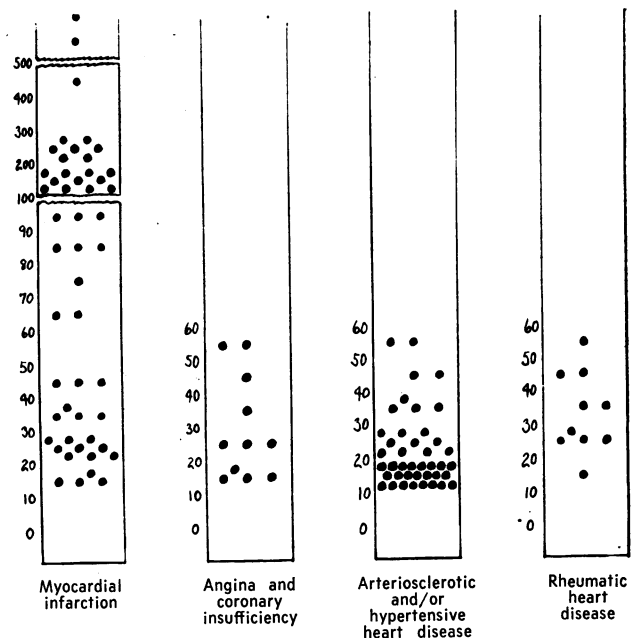


Fig. 1.—Transaminase values in 112 patients with various types of heart disease. Each dot represents the highest value obtained in a single patient. Numbers represent SGO-T units/ml.

illustrates our results in 112 patients with various types of heart disease. The following cases illustrate some of our findings.

CASE 1.—J.L., a 46-year-old man, was admitted on November 21, 1956, with a history of recurring severe retrosternal pains for 24 hours. The pains frequently radiated to the arms and were accompanied by sweating, nausea and vomiting. Over the preceding three weeks he had suffered from the occasional anginal type of pain. Examination revealed him to be acutely ill, dyspnoic and sweating. B.P. 140/100 mm. Hg, pulse 75. The heart was enlarged to the left, and moist rales were present over the right lung base.

On November 23 a pericardial friction rub was noted and the temperature reached its maximum of 103° F.

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