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IDENTIFICATION OF TYPES AND PRIMARY SITES OF METASTATIC TUMORS FROM EXFOLIATED CELLS IN SEROUS FLUIDS *

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THE PROBLEM

It is well established that cells from malignant tumors can be readily recognized as such in smears or cell-block preparations of serous fluids; in only about 10 per cent of the examinations will there be any doubt as to the validity of the diagnosis.[1-7] In this article we are dealing with a somewhat different and more special problem: Can the primary site and type of the tumor from which cells have exfoliated also be deduced with any degree of accuracy when the diagnosis is based upon their cytologic characteristics? It is often difficult or impossible to determine these points by examining stained sections of a metastasis in a lymph node or other tissue. How much more difficult, then, will it be to make such a diagnosis on the sole basis of appearance of cells, or clusters of cells suspended in a serous fluid? Offhand, one would be very doubtful as to the accuracy of the results of such an examination. Consequently the work reported in this paper was undertaken in an attempt to confirm or to dispel such doubt.

Earlier investigations on the identification of types of pulmonary cancer in smears of sputum and bronchial aspirates[8] yielded sufficiently encouraging results to afford considerable stimulation to the investigation of serous fluids.

The results of the present experiment are interesting since they show that, while the accuracy of diagnosis of types and sites made on a series of smears taken consecutively as they come into the laboratory is admittedly poor, it is rather the reverse in so far as the more common tumors comprised in the series are concerned. Five or six kinds

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of malignant tumors constitute 80 per cent of the lot and the diagnosis of primary site and type in this group averages about 68 per cent correct, but the remaining 20 per cent show an accuracy of diagnosis that averages only 18 per cent. While an average of 68 per cent is worth while considering, the over-all average of 45 per cent would discourage further investigation if one did not know why it is so low. It will be shown, however, that there is an encouraging side to the figures; the diagnosis of mammary, bronchogenic, and gastric carcinoma, and of the group commonly known as "the lymphomas," is 70 per cent correct, and these tumors constitute more than half the series (64 per cent). The only other tumor largely represented is ovarian carcinoma (16.4 per cent of series), in which the degree of accuracy is only 58 per cent, too low to be very encouraging. Thus, if it be known which types can be diagnosed with satisfactory accuracy and which cannot, something can be accomplished in attacking the problem.

Of course, there is no hope of taking preventive or curative measures when a patient has generalized metastasis so severe as to flood the serous cavities with malignant neoplastic cells; the outlook is hopeless. Nevertheless, there are inflammatory and mechanical conditions that provoke serous exudates and it often happens that these are mistaken for the manifestations of the presence of metastatic tumor. It is distinctly worth while to rule out the latter and equally so to be in a position to state that the process is frankly inflammatory or mechanical. While this paper is possibly more interesting from an academic than from a practical standpoint, it will bring out definite conclusions that are offered at face value.

Material

The material comprised smears from serous fluids from the pleural, peritoneal, and pericardial cavities of 219 patients. In order to set up this series it was necessary to tap a series of 2,027 reports, as only those could be utilized in which a positive diagnosis of cancer had been made on the smears and, furthermore, the primary site had been established on the basis of necropsy, biopsy, or a definite history pointing to the recurrence of a previously diagnosed malignant tumor.

A preliminary diagnostic run was made on the first 100 of the 219 specimens and, after checking the results for correctness, the smears were carefully reviewed and those in which the diagnosis had been erroneous were studied with particular care to determine whether the average accuracy of diagnosis could not be improved through the discovery of additional diagnostic criteria. It was found that the results of the second run or review of the material yielded approximately the

same number of successes and failures in accurate diagnosis. The remaining 119 smears were next examined and diagnosed and the results pooled with those of the preliminary survey. A summary of the series will be found in Table I.

Report on the Diagnosis of the More Common Tumors

It is best to discuss the results in detail where the more common tumors are concerned and these will be taken up in the order of their frequency of occurrence in the series.

While studying the smears the following points were always kept in mind: (a) the abundance or scarcity of tumor cells in the microscopic field; (b) the size of the cells, their shape, the appearance of their cytoplasm, and the distinctness of their outline; (c) the same factors were observed for the nuclei, and multinucleation was noted when it occurred; (d) the presence of "proliferation spheres" (to be described later) and of abortive structures like tubules was noted also; (e) the presence and type of other, non-neoplastic elements in the smears.

Mammary Carcinoma

Number of cases: 57. Percentage of series: 26.0. Correctly diagnosed: 40 (70.0 per cent). Incorrectly diagnosed: 15 (27.0 per cent). No diagnosis of type possible: 2 (3.0 per cent). Males: 1 (2.0 per cent). Females: 56 (98.0 per cent). Pleural exudates: 51 (89.5 per cent). Pericardial: 1 (1.7 per cent). Peritoneal: 5 (8.8 per cent).

Cytologic Characteristics. The cells of mammary carcinoma are usually not numerous, and are large or medium-sized, spheroidal, and distinctly outlined. Their cytoplasm is either pale and vacuolated, or homogeneous and hyaline, the former type predominating slightly. In all cases the nuclear outline is distinct, the nuclei usually being large and ellipsoidal, less often spheroidal. Their karyoplasm is structured, showing a pattern of karyosomes and threads. Multinucleation is only seldom noted. Very occasionally there may be a suggestion of a double cell-contour. The cells may be arranged concentrically into characteristic spheres which may be called proliferation spheres, as they seem to represent a multiplication of free-lying cells in the fluid. This is a very useful diagnostic feature as it is noted only in mammary and ovarian carcinomas; unfortunately, it is present in only one third to one half of the smears. Otherwise, the arrangement of cells takes the form of disorderly clumps or abortive tubular structures. Smears comprising numbers of discrete cells without any tendency to cluster are a rarity. The non-neoplastic cellular elements in these smears are usually lymphocytes and histiocytes.

TABLE I

Data on Types of Tumor in the Survey

Tumor	No. in series	Percentages				Fluids					
						Pleural		Abdominal		Pericardial	
		In series	Correct diagnosis	Males	Females	No.	%	No.	%	No.	%
Mammary carcinoma	57	26.0	70.0	2.0	98.0	51	89.5	5	8.8	1	1.7
Bronchogenic carcinoma	49	22.4	77.0	77.0	23.0	49	100.0	0	0.0	0	0.0
Ovarian carcinoma	36	16.4	58.0	0.0	100.0	10	27.8	26	72.2	0	0.0
Gastric carcinoma	17	7.8	71.0	47.0	53.0	4	23.5	13	76.5	0	0.0
Intestinal carcinoma	12	5.5	50.0	42.0	58.0	3	25.0	9	75.0	0	0.0
Pancreatic carcinoma	10	4.6	0.0	50.0	50.0	1	10.0	9	90.0	0	0.0
Carcinoma of uterus and cervix	7	3.2	0.0	0.0	100.0	5	71.4	2	28.6	0	0.0
"Lymphoma"	18	8.2	66.7	55.6	44.4	13	72.2	5	27.8	0	0.0
Other tumors	13	5.9	21.3	Irrelevant		8	61.5	5	38.5	0	0.0
Totals	219	100.0	46.0	Average		144	65.7	74	33.8	1	0.5

If the percentages of correct diagnosis be averaged only for the 178 tumors that occurred to the extent of over 7.7 per cent of the series, the low average of 46.0 per cent can be raised to approximately 68 per cent. The text will explain why such a step would not be unreasonable.

Bronchogenic Carcinoma

Number of cases: 49. Percentage of series: 22.4. Correctly diagnosed: 38 (77.0 per cent). Incorrectly diagnosed: 10 (21.0 per cent). No diagnosis of type possible: 1 (2.0 per cent). Males: 38 (77.0 per cent). Females: 11 (23.0 per cent). All of these exudates were of pleural origin.

Cytologic Characteristics. The cells of bronchogenic carcinoma are usually not numerous, and are large and spheroidal, with distinct outline. Their cytoplasm may be pale and vacuolated (in adenocarcinomas) or homogeneous and sometimes slightly keratinized and orangeophile (in the more numerous epidermoid variety). The nuclei are slightly more often large than small, or medium-sized, again depending upon the type of tumor. Sausage-shaped nuclei sometimes are noted, but those with an elliptic shape are more common. Their outline is always sharp and distinct, and their karyoplasm structured in about half the cases. Multinucleation is noted in about 43 per cent of the smears and this is a distinctive feature, as it is seldom noted outside of the bronchogenic group (occasional in ovarian and mammary carcinoma). The cells are usually arranged in *small* groups of from three to eight elements. Proliferation spheres were noted in only three of the 49 cases (6 per cent). Pus is more commonly present in the smears of bronchogenic carcinoma than it is in any of the other groups examined. This is probably attributable to secondary infection from broken-down, cancerous lung.

Ovarian Carcinoma

Number of cases: 36. Percentage of series: 16.4. Correctly diagnosed: 21 (58.0 per cent). Incorrectly diagnosed: 14 (40.0 per cent). No diagnosis of type possible: 1 (2.0 per cent). Pleural exudates: 10 (27.8 per cent). Peritoneal: 26 (72.2 per cent).

Cytologic Characteristics. The cells are usually numerous, large or medium-sized in about equal proportions, and usually spheroidal. Their outlines are distinct when they lie discretely separated in the smear, but, when they are clustered, outlines are so indistinct as to give the clumps a syncytial appearance. In 19 per cent of the smears there was an apparently double contour of the cell-membrane, an outer and distinct ring and an inner one that was too vague to constitute a true double contour like that of blastomyces. However, there is a conspicuously clear border zone that gives an impression of a double contour. The cytoplasm is usually clear, pale, and conspicuously vacuolated; rarely dense with small vacuoles, or variable. The nuclei are usually medium-sized; they are ellipsoidal in three fourths

of the cases and sharply outlined. More than one third show a structured karyoplasm, while one fourth are laked or diffuse. The rest of them are densely staining, almost to the point of pyknosis. Proliferation spheres are very characteristic when present, but unfortunately they occur in only 36 per cent of the smears. They are less concentrically arranged than are those of mammary carcinoma, but they are distinctly outlined by a membrane of peripherally situated cells. Disorderly clusters of variable size are often noted. One smear showed cells grouped into short, abortive, duct-like arrangements. Non-neoplastic elements are chiefly lymphocytes and histiocytes.

Gastric Carcinoma

Number of cases: 17. Percentage of series: 7.8. Correctly diagnosed: 12 (71.0 per cent). Incorrectly diagnosed: 4 (23.0 per cent). No diagnosis of type possible: 1 (6.0 per cent). Males: 8 (47.0 per cent). Females: 9 (53.0 per cent). Pleural exudates: 4 (23.5 per cent). Peritoneal: 13 (76.5 per cent).

Cytologic Characteristics. The cells of gastric carcinoma are usually very sparsely distributed and hard to find. They are small and spheroidal in about one third of the smears and elliptic in 20 per cent. The rest may be angulated, elongated, or sausage-shaped. Slender fusiform cells appear often enough to be very characteristic when present. Cellular outline is always distinct and the cytoplasm is pale and vacuolated in 70 per cent, granular in 20 per cent, in the rest, clear and watery. The nuclei are either large or medium-sized in proportion to the cell, their outline being distinct and ovoid rather than elliptic. Their karyoplasm is structured and rather dense. Cell clusters are small and may take the form of abortive tubules, but they are less numerous than in smears of other tumors. Lymphocytes and histiocytes are the usual non-neoplastic elements.

Lymphoid Tumors

Number of cases: 18 (including 3 of Hodgkin's disease). Percentage of series: 8.2. Correctly diagnosed: 12 (66.7 per cent). Incorrectly diagnosed: 5 (27.8 per cent). No diagnosis of type possible: 1 (5.5 per cent). All of the Hodgkin's disease cases were correctly diagnosed as such. Males: 10 (55.6 per cent). Females: 8 (44.4 per cent).

Cytologic Characteristics. The cells of the lymphoid tumors are usually extremely numerous. They vary in size with the type of lymphoma present (small lymphocytes, large lymphocytes, and, in Hodgkin's disease, large pale cells with vesicular nuclei reminiscent of histiocytes). The variability in the cells in the smears at once arouses

suspicion of a lymphoma, rather than of a lymphocytic inflammatory exudate, in which they would be small and uniform. They present manifest abnormalities in size, shape, and density. It is important that they are evenly distributed throughout the smear as discrete elements and that they do not cluster. They are distinctly more numerous than are the cells of carcinomas. The presence of eosinophilic leukocytes in the exudates of Hodgkin's disease is a very useful and generally available diagnostic feature. Large "Hodgkin's sarcoma cells" may be occasionally noted; however, typical Reed-Sternberg cells are difficult to recognize in smears of serous fluids, although they can be readily found and identified in contact smears from granulomatous lymph nodes.

Other Tumors

Other tumors in the series were not represented sufficiently often to make detailed analysis of their cellular characteristics either feasible or accurate. There were twelve intestinal carcinomas, half of which were correctly diagnosed. Their cells were large, the cytoplasm pale

TABLE II

Data on "Other Tumors" in Last Category of Table I

Tumor	No.	Percentage	Diagnosis	Fluid		Sex	
				Pleural	Abdominal	Male	Female
Rhabdomyosarcoma	2	0.9	None possible	1	1	1	1
Malignant melanoma	2	0.9	Incorrect	2	0	1	1
Mesothelioma	1	0.5	Incorrect	0	1	1	0
Hepatic carcinoma	1	0.5	Incorrect	0	1	0	1
Carcinoma of gallbladder	1	0.5	Incorrect	0	1	0	1
Carcinoma of tongue and esophagus	1	0.5	Correct	1	0	1	0
Transitional cell carcinoma of bladder	1	0.5	Incorrect	1	0	1	0
Carcinoma of buccal mucosa	1	0.5	Correct	1	0	1	0
Wilms' tumor	1	0.5	Incorrect	0	1	0	1
Liposarcoma	1	0.5	Incorrect	1	0	1	0
Osteosarcoma	1	0.5	Incorrect	1	0	1	0
Totals	13	5.9		8	5	8	5

The two "correct" diagnoses were correct only in so far as they recognized highly keratinized epidermoid carcinoma, the primary site of which was conjectural.

and vacuolated, and their nuclei vesicular. The arrangement of the cells was irregular, but sometimes suggested the attempted formation of tiny papillae. This is, however, a mere list of equivocal features and the resulting 50 per cent in accuracy is no better than a random guess.

Nothing was noted from pancreatic, uterine, or intestinal tumors

TABLE III

Summary of Cellular Characteristics in Five Groups of Malignant Tumors

Tumor	Cells			Cytoplasm	Double contour	Nuclei			Multinucleation	Proliferation spheres	Tubules
	Number	Size	Shape			Size	Shape	Karyoplasm			
Mammary carcinoma	Few	L	Sphr.	Var.	No	L	Var.	Struc.	Occasional	Yes	Occasional
Bronchogenic carcinoma	Usually few	L	Sphr.	Var.	No	L	Ellip.	Struc.	Yes	No	No
Ovarian carcinoma	Numerous	L–M	Sphr.	P & V	Yes	M	Ellip.	Var.	Occasional	Yes	Seldom
Gastric carcinoma	Few	Small	Var.	P & V	No	L	Oval	Struc.	No	No	Yes
Lymphoid	Very numerous	Var.	Sphr.	Var.	No	L	Sphr.	Struc.	No	No	No

L = large, M = medium, Var. = variable, Sphr. = spheroidal, Ellip. = ellipsoidal, Struc. = structured (showing a pattern of karyosomes and filaments), P & V = pale and vacuolated.

that could be considered to be even reasonably diagnostic. As for the two myosarcomas, their cells were completely primitive and in no way suggested myoblasts. The cells in the two instances of malignant melanoma so seldom showed any intracellular pigment that the diagnosis was missed. One smear did present prominently pigmented cells that could be recognized as probable melanoblasts, but this was a single smear from a number of negative examples. While it is possible that mesothelioma might be diagnosed on the basis of a serrated outline to its cells (produced by a peripheral ring of small vacuoles), this feature was absent in the one case in this series and the diagnosis was missed.

Sundry Observations on the Survey

Fluids. There were 144 pleural, 74 peritoneal, and one pericardial fluid in the collection of smears; this represents percentages of 65.7, 33.8, and 0.5 respectively. The reason for the preponderance of pleural exudates can best be explained on the basis of the site of the majority of tumors in the set. Mammary and bronchogenic carcinomas together comprised 48.4 per cent of the cases and several of the other types of carcinoma exhibited a surprising predilection for intrathoracic metastasis; for instance, 72.2 per cent of the lymphomas and 27.8 per cent of the ovarian carcinomas were submitted as smears of pleural effusions.

Importance of Clinical Data. From what has been said, it might appear that the diagnosis of types and sites of tumors was based purely on objective data. While the study was purposely kept as objective as possible, it would be a mistake to observe this objectivity while making routine examinations in the laboratory. Any diagnostician is entitled to know all the facts about his case and a knowledge of the clinical history of each is imperative. The source of the fluid, the sex of the patient, and his past clinical history will make the reading of the smears far easier. To illustrate: let us postulate that a smear of fluid is examined and diagnosed objectively; the cellular characteristics of the specimen will give certain clues which will be sufficient to enable the cytologist to make a fairly positive diagnosis. But if the data submitted with the specimen indicate that the fluid is pleural, that the patient is an elderly woman whose left breast was removed 5 years earlier for carcinoma, the diagnosis becomes well rounded out. Should the results of cytologic examination and the clinical history be at serious odds, it will behoove the clinician to search for a possible second tumor in the case, for the occurrence of multiple primary malignant tumors of different sorts is not uncommon.

Summary and Conclusions

It is possible to diagnose the type and source of malignant tumor cells in serous effusions with an over-all accuracy of about 50 per cent. This figure may be greatly improved by reckoning only those tumors which are most commonly found in a series of smears of such effusions, such as mammary, bronchogenic, and gastric carcinoma and "lymphomas" of various types.

The degree of accuracy is influenced largely unfavorably by the fact that ovarian carcinomas (which rate as "numerous" in such a series) cannot be more accurately recognized. They often invade the pleura (which is misleading) and they may be quite equivocal in regard to their cytologic features. Tumors less commonly found in smears of serous fluids, such as sarcomas, mesotheliomas, and melanomas, are fortunately so few in number that mistakes in diagnosis need not discourage us. If the majority of tumors can be diagnosed with a degree of from 65 to 76 per cent of accuracy, the method is surely worthy of consideration and of further study.

All photomicrographs were taken by Miss Suzanne Voorhies of the Department Staff.

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LEGENDS FOR FIGURES

Figs. 1 to 7 are examples of cells from mammary carcinoma.

FIG. 1. Single "proliferation sphere," typical of mammary carcinoma. × 600.

FIG. 2. A pair of proliferation spheres, showing the concentric arrangement of cells. × 600.

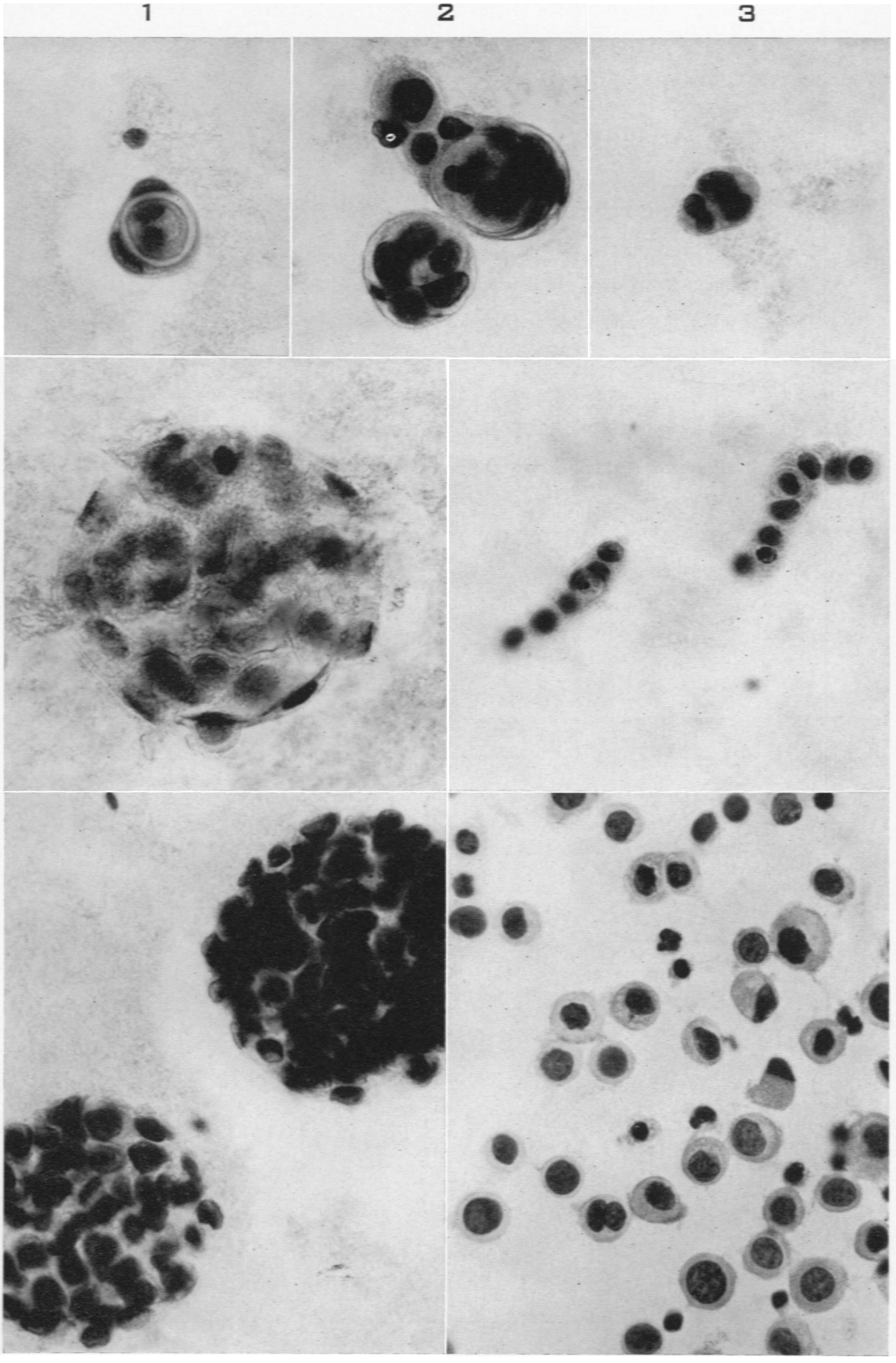
FIG. 3. Proliferation sphere in early stage of formation. × 600.

FIG. 4. A larger and differently constituted proliferation sphere. × 600.

FIG. 5. Two chains of cells suggesting the scirrhous form of carcinoma. × 600.

FIG. 6. Two large, simple clusters similar to those often seen in ovarian carcinoma. × 600.

FIG. 7. Unusually copious exfoliation, one cell binucleate. × 600.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Figs. 8 to 15 are examples of cells from bronchogenic carcinoma.

FIG. 8. Heavy exfoliation of an epidermoid bronchogenic carcinoma. × 600.

FIG. 9. Single keratinized cell surrounded by pus cells, epidermoid type. × 600.

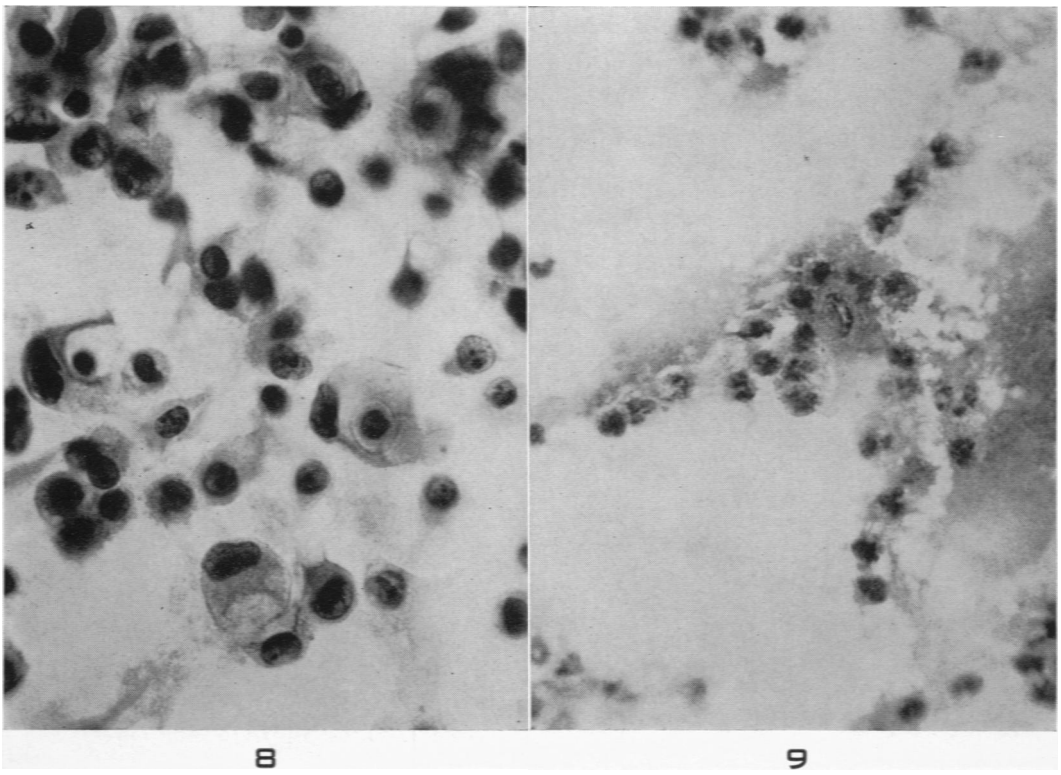
FIGS. 10 and 11. Examples of multinucleation in this type of carcinoma. × 600.

FIG. 12. Cluster strongly indicating anaplastic or oat-celled type. × 600.

FIG. 13. Vacuolated cells very typical of bronchogenic adenocarcinoma. × 600.

FIG. 14. Cells probably indicating pleomorphic bronchogenic carcinoma. × 600.

FIG. 15. A cluster suggesting terminal bronchiolar origin. × 600.



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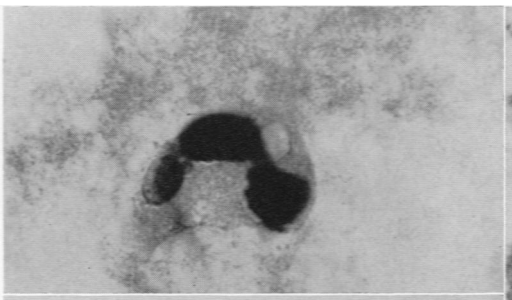

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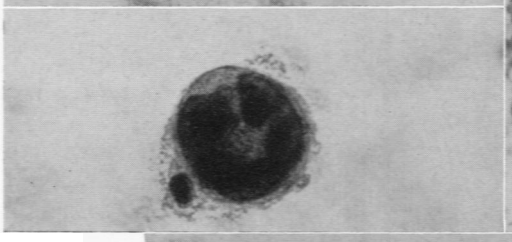

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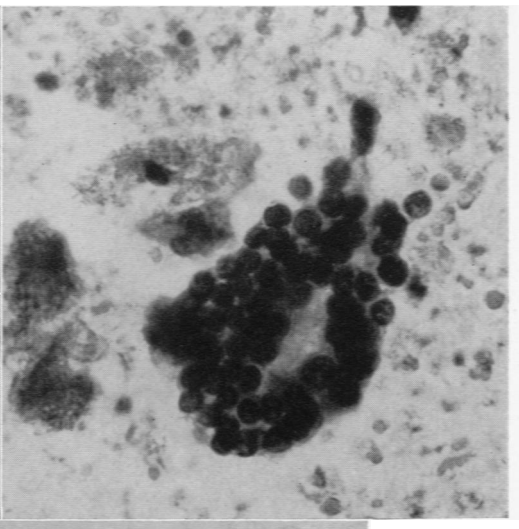

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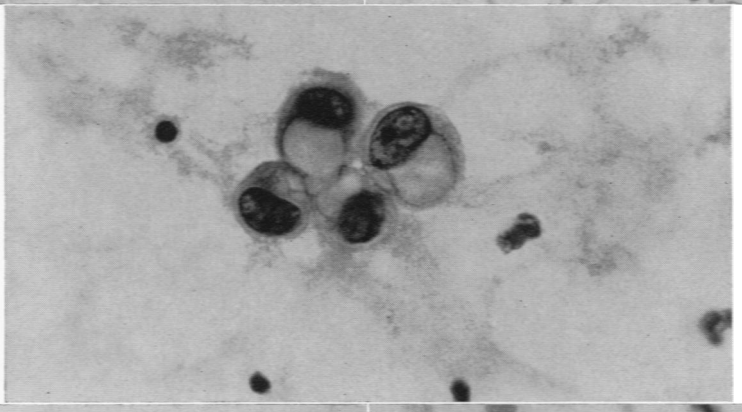

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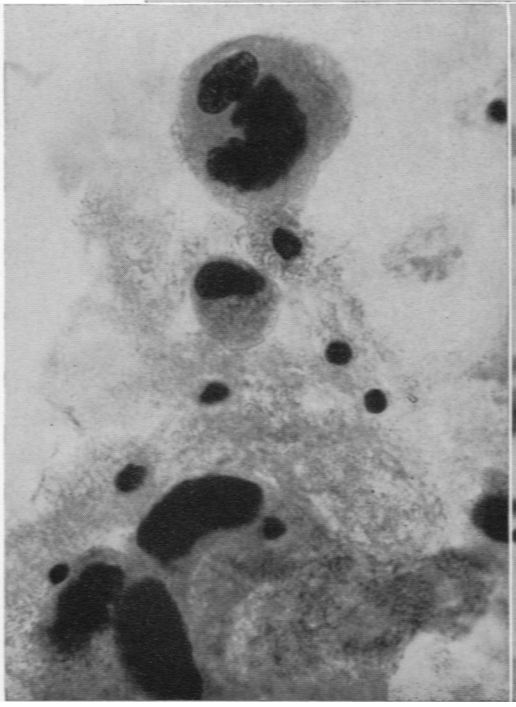

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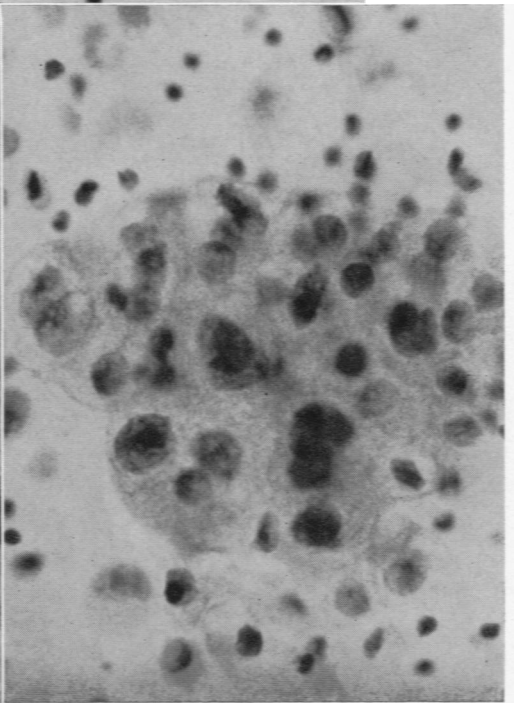


Figs. 16 to 20 are examples of cells from ovarian carcinoma. Figs. 21 to 24 are examples of cells from gastric carcinoma.

FIG. 16. Papilliform proliferation sphere. × 600.

FIG. 17. Cluster illustrating sham double contour of cells. × 600.

FIG. 18. Large binucleate signet-ring cell, unfortunately rarely found in smears of serous fluids. × 600.

FIG. 19. Finely vacuolated cells in copious exfoliation. × 600.

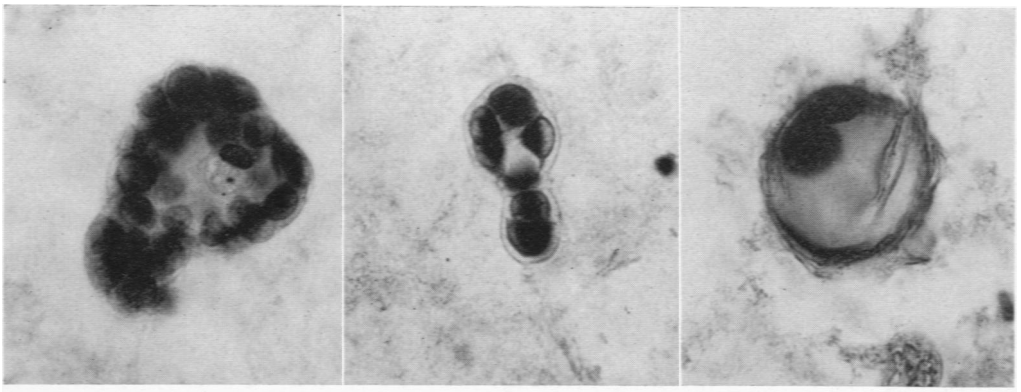
FIG. 20. Nondescript cells too often found in smears of ovarian carcinoma. They account for many mistakes in diagnosing source. × 600.

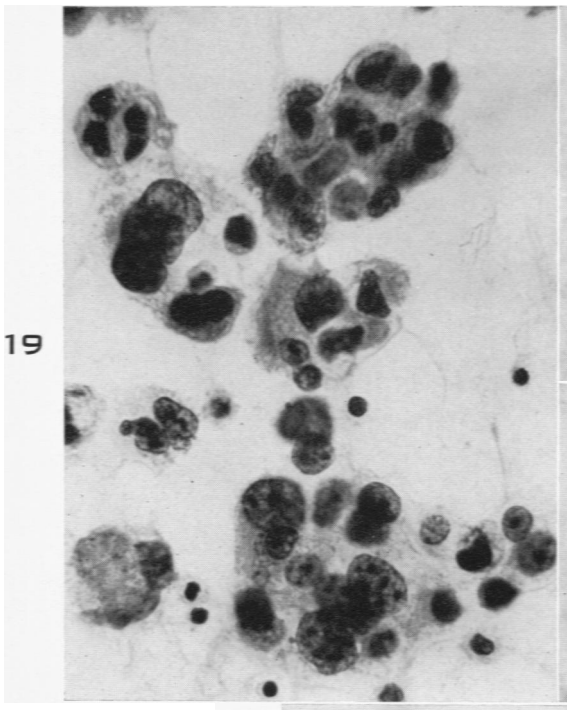
FIG. 21. Cluster of small typical cells from gastric carcinoma. The nuclei are almost pyknotic. × 600.

FIG. 22. Enormous vacuolated cell. × 600.

FIG. 23. Fusiform epithelial cells noted only in gastric carcinomas in this series. × 600.

FIG. 24. Sausage-shaped cells. × 600.





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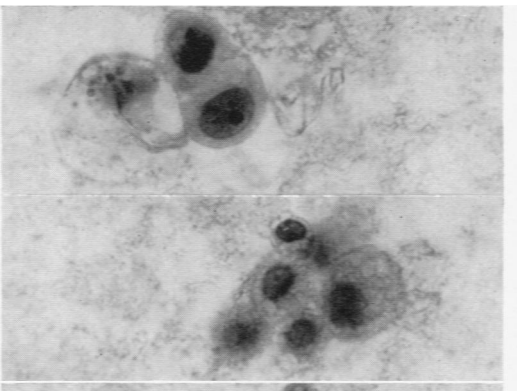

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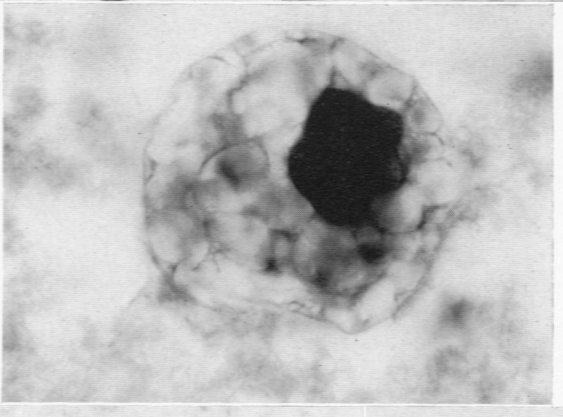

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Fig. 25 is from a smear of gastric carcinoma.

FIG. 25. Proliferation sphere that is a rarity in gastric carcinoma and would probably cause a mistaken diagnosis of mammary or ovarian carcinoma. × 600.

Figs. 26 to 28 show cells from cases of lymphoma.

FIG. 26. Typical appearance of cells of Hodgkin's disease. Of note are the pallor of the cells and their size as compared with lymphocytes. × 600.

FIG. 27. Typically copious exfoliation in lymphosarcoma. × 600.

FIG. 28. Larger lymphoid cells from lymphosarcoma. × 600.

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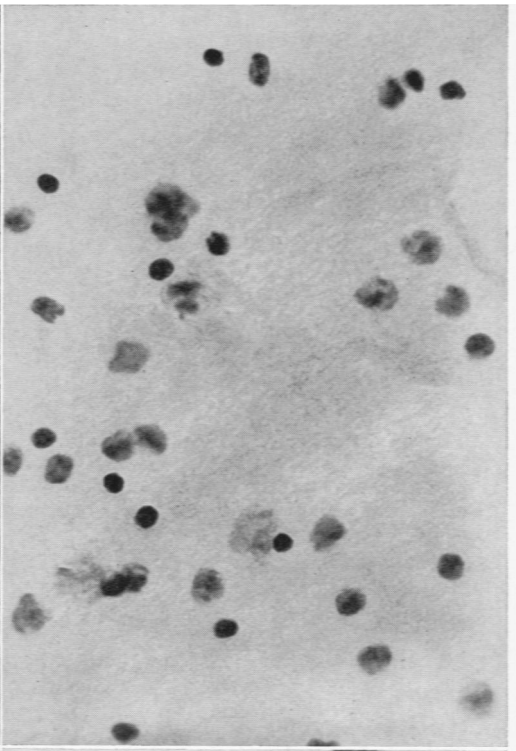

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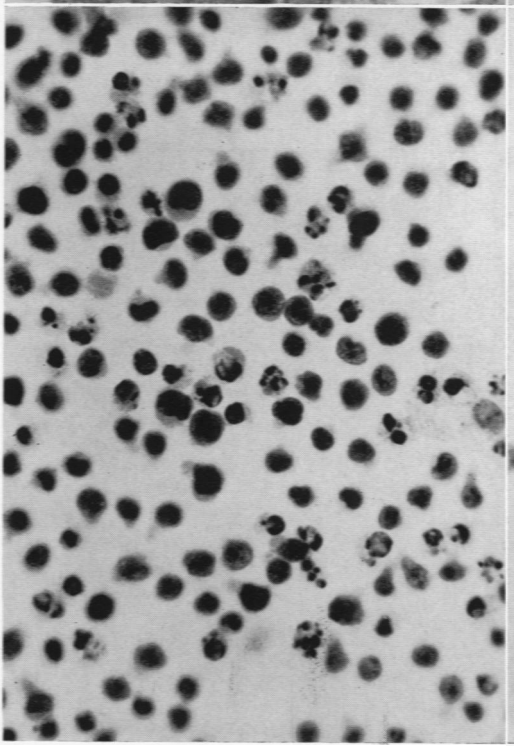

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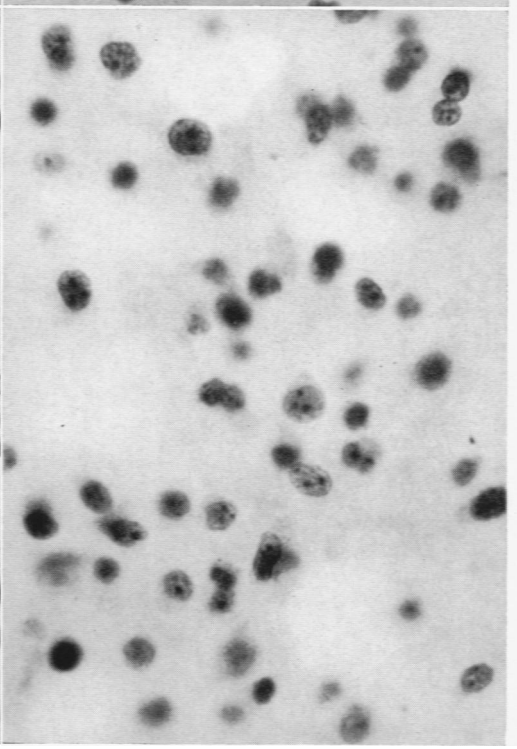

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