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THE IDENTIFICATION OF TUMOR CELLS IN SEDIMENTS OF SEROUS EFFUSIONS *

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Microscopic examination of sediments from aspirated effusions, chiefly those of pleural or peritoneal origin, has proved to be a useful procedure in the clinical diagnosis of primary and metastatic malignant tumors of the membranes of these cavities. As such examinations frequently cause considerable discussion and offer many difficulties in the way of exact diagnosis, the following paper is presented in an attempt to evaluate our criteria for judging whether or not cells of malignant tumors are present in a given specimen. In our experience these examinations have been of most value in connection with exudates from the pleura, pericardium and peritoneum; gastric contents, sputa and the like have not proved to be favorable material on account of the mucous content, the paucity of cells in a given sample, and so on. As Graham [1] has reported in a similar article, many hospital pathologists have resorted to essentially the same technique for treating and examining these sediments, devising their methods quite independently of one another; probably the first to publish such a procedure was Mandlebaum [2] in 1917. Smears of fluid sediments have been examined since the earliest days of pathology; Zemansky [3] gives an excellent review of the history of this technique. Four articles have recently appeared on the examination of smears stained in various ways, the most modern employing vital, or supravital technique. These papers are by Quensel,[4,5] Karp,[6] and Merklen, Waitz and Kabaker [7]: the first two will be re-

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ferred to later on. Merklen, Waitz and Kabaker have published an excellent study of the cytological varieties and peculiarities of cells found in serous effusions, illustrated by a large colored plate that is valuable as an atlas to those inexperienced in the examination of these fluids.

The method devised by Mandlebaum consists of allowing the specimen to settle somewhat, decanting the supernatant fluid and centrifugating the residue until a small button forms at the bottom of the tube. This is then isolated by pouring off the fluid and replacing it with fixing solution which shrinks the button and separates it from the glass. It is then removed and cut in two vertically, so that its section surface represents all the layers of the centrifugate. It is then embedded in paraffin. Any fixing fluid may be used and the sections cut from the button may be stained as desired. During the past 3 years we have used essentially the same technique. If the amount of fluid be small, it is immediately centrifugated; if there be a large quantity enough glacial acetic acid is added to bring the acid percentage up to about 2 per cent (20–30 cc. per liter). This is stirred in and prevents the coagulation that ruins one's chances of obtaining a good sediment; it also lakes the excess of erythrocytes and thus rids the sediment of much confusing detail. The fluid is set in the ice-box overnight, the clear supernatant fluid decanted, and the cloudy residue is centrifugated until the button is well formed. Our routine fixative for this is 10 per cent formalin in 95 per cent alcohol. Mandlebaum's method of bisecting the button vertically is good in theory, but in practice we have found that it tends to crumble to bits during the process and that it is better to embed it on its side and cut well into it on the microtome before taking off paraffin sections. These are best stained by Masson's trichrome light green method, which gives excellent details and usually stains the nucleoli vermillion with the ponceau, so that they are readily identified and measured. The same dye is excellent for demonstrating intercellular bridges, which is often of diagnostic advantage. Any other stain is, of course, applicable.

Zemansky has established some criteria for judging the presence or absence of tumor cells in a given fluid. He states that tumor may be suspected when one finds: (1) fragments of tissue with definite arrangement of cells and stroma, such as acini or papillae; (2) multiple groups of large, deeply staining cells, giving the section a

mottled appearance; and (3) finer cellular changes, such as extreme irregularity of cell outline, eccentricity of nucleus, extremely large size of nucleus, multinucleation and typical or atypical mitotic figures. He believes that it is all-important to know the duration of the effusion and the history of the case, as the longer the duration the more atypical will be the cells observed. He has not found mitotic figures in fluids not showing tumor cells, although he notes that Dock reported finding them. His review of the accuracy of the method, as checked by autopsy and biopsy at the Mount Sinai Hospital in New York over a period of many years, shows that the percentage of correct diagnoses was 87 per cent in the case of positive fluids, and 47 per cent in that of fluids reported negative. Fluids from carcinoma cases showed cells in 60 per cent of 55, and those from sarcomas 33 per cent of the total examined. Of 35 cases of intra-abdominal neoplasm, 65 per cent showed tumor cells in the fluid, and 30 cases of intrathoracic tumor showed 50 per cent.

Curious to ascertain how correct our results might have been, I have reviewed all our material received between September 1932 and January 1936, consisting of 55 specimens of ascitic fluid and 85 of pleural effusion. Four pericardial fluids were all negative and will not be discussed. Of the pleural and ascitic fluids three patients furnished 5 specimens each, two 4, five 3 and eleven 2 apiece; the remainder furnished a single specimen each. In reviewing this admittedly small series of specimens the results obtained at the original routine examinations were collected and checked for accuracy against the respective histories, using any available data from autopsy, biopsy, operation, X-ray examination or, failing these, from the clinical history to guide us. All these sections were next examined on a purely morphological basis, without any knowledge of whence they came or what may have been the history in each case.

The first series, diagnosed by the staff and myself in collaboration, showed 62 per cent correct, 34 per cent incorrect and 4 per cent doubtful in the ascitic fluid cases; the recheck, done on a purely morphological basis, showed 72 per cent correct, 24 per cent incorrect and 4 per cent doubtful. The original reports on pleural fluids were 70 per cent correct, 26.5 per cent incorrect and 3.5 per cent doubtful; my recheck was only 65 per cent correct, 31.5 per cent incorrect and 3.5 per cent doubtful. This was largely due to extreme caution in pronouncing doubtful cells to be tumor cells.

In making the recheck each slide was examined and plus or minus signs were placed in columns under the headings "tumor," "mitoses," "multinucleation," "metaplasia," "cell clumps," and "acini or papillae." Mitoses were present in 26 per cent, multinucleation in 65 per cent, metaplasia in 43.5 per cent, clumping in 54 per cent, and acini or papillae in 15 per cent. Forty-eight cases had histories of proved carcinoma. If we check these against these headings we find that the number of examinations in which multinucleation, metaplasia and cell clumping were found exceeds the total number of proved cases (35 per cent) and that these criteria are not reliable for judging the presence or absence of tumor cells. Mesothelial cells floating in a transudate, which is apparently an excellent culture medium, multiply by mitotic division and round up and become at least anaplastic in their appearance, which is confusing. Mitoses were seen in 24 positive and 12 negative fluids, so that their value as an indication of the presence of neoplasia is about 66 per cent; atypical, or "monster" mitoses may, however, be considered to be pathognomonic of the presence of tumor cells.

Before comparing our figures with those of Zemansky, which were tabulated on a somewhat different basis, those cases not checked by anything more accurate than clinical data were discarded and the rest were then tabulated. In the original series of reports the diagnosis of ascitic fluids that should have shown tumor was 73 per cent correct; in the recheck it fell to 60 per cent. Ascitic fluids with no proof of carcinoma were correctly diagnosed in only 36 per cent of the original reports, while the recheck improved this to 75 per cent. The original diagnoses on pleural fluids from cases with proved cancer were 60 per cent; the recheck fell to 53 per cent. Pleural effusions from cancer-free cases (of which there were only 3) were correctly diagnosed in both series. Averaging these results to cover all these cases selected for comparison with Zemansky's figures, it is found that 66.5 per cent are correct in positive tumor cases and 68 per cent in tumor-free cases in the original series; in the recheck 56.6 per cent are correct in the positive and 87 per cent on the tumor-free cases. The results, then, fall far below Zemansky's 87 per cent where tumors are concerned, but lie well above them where the reports are negative, his figure being 47 per cent. Table I in the text will show a compilation of these figures, together with those obtained

TABLE I

Percentage of Correct and Incorrect Diagnoses of Presence of Tumor Cells

General Results on Entire Series (Unselected Cases)

Source	1st Examination			2nd Examination			3rd Examination		Averages		
	C	I	D	C	I	D	C	I	C	I	D
Abdominal Fluid	62	34	4	72	24	4	63	37	65.5	31.5	3
Thoracic Fluid	70	26.5	3.5	65	31.5	3.5	70	30	68.5	29.5	2

Results on Selected Cases Checked by Autopsy, Biopsy or Operation

Tumor Present											
Abdominal Fluid	73	27	0	60	40	0	72	28	69	31	0
Thoracic Fluid	60	40	0	53	47	0	81	19	65	35	0
Tumor Absent											
Abdominal Fluid	36	64	0	75	25	0	62	38	58	42	0
Thoracic Fluid	100	0	0	100	0	0	58	42	89	11	0
Average of selected series									70	30	0
Average of both series									68	30.5	1.5

C = correct; I = incorrect; D = doubtful.

when using another method of attack on the problem, which will be discussed presently.

The reason for mistakes in diagnoses of this sort is not far to seek: there are large numbers of exfoliated mesothelial cells in most fluids of long standing, whether of quasi-inflammatory origin, or provoked by the presence of tumors or their metastases. These cells often resemble those of malignant growths quite strikingly in that they present hyperchromasia, large and deeply stained nuclei and often nucleoli, are frequently multinucleated, quite often show mitotic figures, clump together to form structures much like acini and often show eccentrically placed nuclei. One or two disastrous experiences in misdiagnosing these cells as cancer cells tend to make the observer unduly cautious and pessimistic. For these reasons, Zemansky's criteria must be revised. We may accept the presence of frank tumor fragments as incontrovertible. Irregularity in the size and shape of the nucleus and the presence of large and bizarre forms or *atypical* mitotic figures are also criteria that stand confirmed.

What further data can one adduce to assist in improving one's accuracy of diagnosis in these instances? Quensel [4,5] has devoted much time to the origin of the various cell forms encountered in smears of sediments and appends a good bibliography to his copiously illustrated papers. He stresses the point that the ratio of nucleus to nucleolus is always high in cells of malignant tumors and much lower in the normal components of mesothelial or retothelial tissues. In the former it lies between 0.20 and 0.40, in the latter below 0.20. He obtains this ratio by measuring the diameters of nucleus (N) and nucleolus (n) and dividing the latter by the former. We shall speak of this ratio as the "n/N ratio" in this paper. He prefers moist smears of sediments stained supravitally with methylene blue cadmium or Sudan III cadmium, but has also used Giemsa's and Ehrlich's triacid stains. Following his lead, Karp [6] has worked along similar lines and confirmed Quensel's findings. Believing that accuracy was to be improved by using the ratio of areas, rather than diameters, he measures the longest and shortest diameters of nucleus and nucleolus and calculates their areas from the formula for obtaining the area of an ellipse $\pi \left(\frac{d_1 + d_2}{4}\right)^2$ applied to each set of diameters. The figures thus obtained may be more

accurate but they improve the results relatively little, the process is time-consuming, and the fact that cells in fluids tend to become rounded up into spheroids makes it rather unnecessary. There are comparatively few elongated ellipsoidal cells to deal with.

Forty-four representative slides were segregated from our series and I applied Quensel's measurements and method for obtaining the n/N ratio to each, without using any other criteria whatsoever. Ten cells were measured with an ocular micrometer in each section and the diameters averaged, the n/N ratio being calculated by dividing the average nucleolar by the average nuclear measurement. The figures, obtained in fixed and embedded sediments, agree entirely with those of Quensel and of Karp and the percentage of accuracy of diagnosis arrived at by this method is surprisingly good.

It is found that in making these measurements only doubtful cells should be selected, otherwise the large number of monocytes and frankly mesothelial cells present will bring the ratio too low, where only a few tumor cells are present. A typical ascitic fluid, for example, will show a majority of cells with nucleoli too small to be accurately measured with a No. 3 ocular micrometer and a 1/12th oil immersion objective. In such cases one may be reasonably sure that no neoplastic elements are present. When, however, a small number of cells with prominent nucleoli are seen, one should measure these. One of our sections, about to be pronounced non-neoplastic, was shown to be the contrary because the doubtful cells gave a high n/N ratio. Further search revealed a large clump of typical carcinoma cells in a tumor fragment, which appeared in only one out of three serial sections.

Most of the fluids show a ratio below 0.20 when no tumor is present, but one of 0.25 to 0.40 when it is present, which is in material accord with Quensel's and Karp's figures. The disappointing feature of this method proves to be a small group of cases whose n/N ratio falls between 0.20 and 0.25, for it is just this group that causes doubt when judged on a morphological basis. The average in twenty-one sections from pleural and ascitic fluids diagnosed as tumor-positive was 0.265, the ratios running from 0.17 to 0.40; pleural effusions from 8 cases of cardiac decompensation gave an average n/N ratio of 0.204, fluctuating between 0.15 and 0.23, and the purely ascitic fluids averaged 0.207 with a low of 0.16 and a high of 0.28. Out of these 44 sections 68 per cent were correctly and 32

per cent incorrectly diagnosed by the use of the n/N ratio, which is a hopeful sign as an attempt was made to neglect morphology utterly and to rely solely on the n/N ratio.

These findings are of interest in view of what MacCarty [8, 9] has claimed for several years as to the difference in the ratio between the size of nucleus and nucleolus in cells of hyperplastic normal tissues and those of neoplasms. He has insisted on these measurements being made in fresh material stained without fixing. In his most recent paper he has tabulated the results obtained by other observers who have followed his methods and one is struck by their general agreement. These workers all use the N/n ratio, which is the reverse of that used by Quensel, Karp and myself, in that the nuclear measurements are divided by the nucleolar, the greater by the lesser. They also work on a volumetric, rather than on a diametric or areal basis, taking the longest and shortest diameters for their areal calculation and multiplying the result by the shortest diameter, on the assumption that nucleus and nucleolus have a single long diameter and two equal transverse diameters. A little calculation on the part of the reader will show that the ratios they obtain are quite similar to those arrived at in this paper. An n/N ratio of 0.15 equals an N/n ratio of 6.66, one of 0.20 (the "critical point") will be 5.0, one of 0.25 will be 4.0 and our maximum of 0.40 will become 2.5 in the N/n figures; by multiplying these N/n ratios to obtain the cubes, figures are found that tally quite closely with those in MacCarty's paper.[9] One cannot figure out the actual spheroidal volume from these diametric ratios without knowing the longest and shortest diameters, for reasons already given.

In a recent paper Guttman and Halpern [10] have reported the examination of a large number of tumor and normal hyperplastic cells in tissues fixed in Zenker's solution and stained by Pappenheim's method. Their findings prove to their satisfaction that there is no appreciable difference between the N/n ratios of these two groups. Be this as it may, the results set down in this paper would indicate strongly that there is a definite difference in the case of cells in effusions, whether fixed or unfixed tissue is examined. MacCarty makes an exception in the case of two types of cell: the oöcyte and the cells of the nervous system, such as ganglion cells. In the case of sediments from effusions it might be well to except the cells of the lymphocyte series from the general rules here set down; their

nucleoli are too inconspicuous to measure. Large numbers of these cells, however, indicate clearly enough some dyscrasia in the lymphoid system if they be predominatingly immature in their type.

It is found that the presence of "signet ring cells," stressed by some authors as indicative of neoplasia, is a relatively common finding; the vacuoles in macrophages often mislead one into mistaking them for cells of a mucous carcinoma. This doubt may be readily settled by using a mucicarmine stain, which will show mucus as a bright vermillion substance in such vacuoles. Mesothelial cells often have a peripheral border-zone of small vacuoles, or projecting serrations that suggest intercellular processes, facts that may be of help in identifying them.

Four photomicrographs have been taken to illustrate this article. Figure 1 shows a section of sediment from the pleural fluid in a case of carcinoma of the ovary; almost anyone could diagnose this correctly at a glance. Figure 2 came from the pleural fluid in a case of carcinoma of a stem bronchus with pulmonary metastasis. The clumps of large dark cells with apparently prominent nucleoli are significant, but without the n/N ratio (0.266 in this case) one might be left in doubt. In Figure 3 we have a sediment from an ascitic fluid in a case that was later examined by autopsy and no tumor was found. This case gave us much trouble as it was repeatedly reported positive for tumor on the original examination of five consecutive specimens. On the recheck, however, it was correctly diagnosed in every instance. The n/N count gave four out of five correct diagnoses, the ratio being 0.193 in the specimen illustrated, which indicates "no tumor found." As Zemansky points out, a negative diagnosis is inconclusive, as tumor might be present without any cells getting into the fluid. Here, however, autopsy showed that there was no tumor. Figure 4 is from a chest fluid from a case of cardiac decompensation with terminal pulmonary infarcts. This also gave us trouble. There are numerous large cells present, one of them showing a normal mitosis with slender chromosomes. The n/N ratio here is 0.172, which is definitely low.

Additional typical photomicrographs might have been selected for publication, but there are plenty of them in the literature; Quensel's articles have 35 plates and 74 figures which illustrate every conceivable phase of the question in excellent photomicrographs.

Summary and Conclusions

Summarizing the findings of this brief investigation we should revise the criteria for reporting "tumor cells present" in a given fluid in somewhat the following manner:

1. Zemansky's first criterion, the presence of fragments of tumor with the cells arranged in acini or papillae about a stroma that is definitely fibrovascular, stands uncontroverted.

2. A nucleolar-nuclear ratio falling above 0.25 is of undoubted value; one of 0.30 or more practically pathognomonic of the presence of tumor.

3. Mesothelial pleural, pericardial and peritoneal covering cells present the chief obstacle in the way of successful diagnosis as they are readily confused with tumor cells on account of their large size and prominent nucleus. When they are measured the dimensions are found to be quite uniform and regular; tumor cells, on the other hand, show a high n/N ratio and a wide variation in measurements.

4. Multinucleation is of no diagnostic value and cell clumping is almost as worthless. Mitosis occurs in both positive and negative sediments, but monster, or abnormal mitoses are found only in tumor cells.

5. The occurrence of erythrocytes and fibrin is of little diagnostic value.

Note: I am much indebted to Dr. Earl P. Lasher for his assistance in compiling the histories and assembling the material for study in connection with this paper, and to Miss E. Dreyfoos, of the department of photography, for the photomicrographs.

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DESCRIPTION OF PLATE

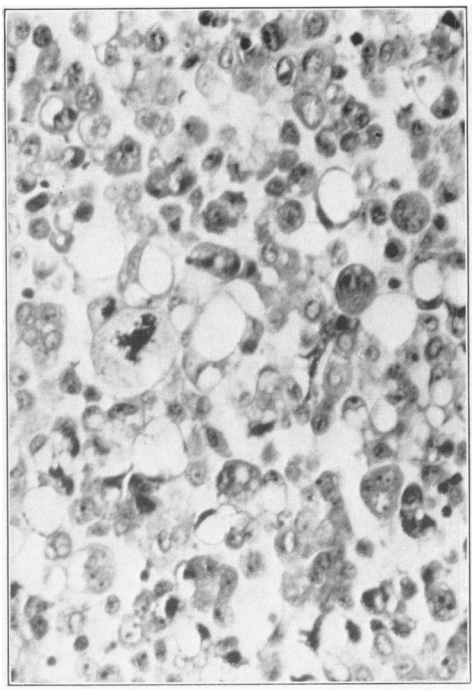
PLATE 1

FIG. 1. Sediment from a chest fluid in a case of ovarian carcinoma. Note the irregularity in size and shape of the cells, the enormous mitotic figure in the center of the field and the prominent nucleoli. × 327.6.

FIG. 2. Sediment from a chest fluid from a case of main stem bronchial carcinoma with pulmonary metastases. This is much less evidently neoplastic, but the irregularity of the cells is manifest and the nucleoli are prominent. × 327.6.

FIG. 3. Sediment from ascitic fluid in a case of periportal cirrhosis of the liver. The cells are numerous, deeply staining and apparently neoplastic. Mitoses are present elsewhere in the section, not shown in this field. × 327.6.

FIG. 4. Sediment from pleural fluid in a case of decompensated heart and pulmonary infarcts. A large mesothelial cell in mitosis is shown at the center. Notice occasional clumping of the cells. × 327.6.



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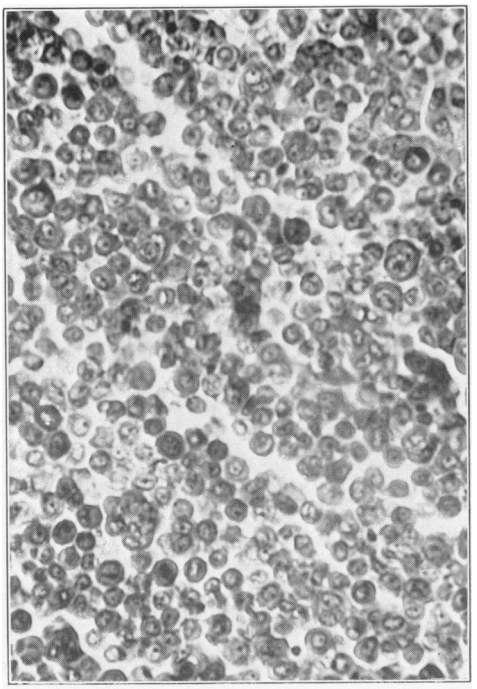

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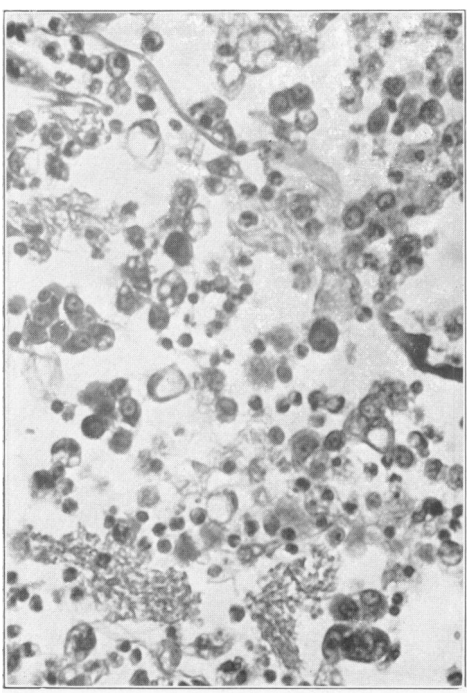

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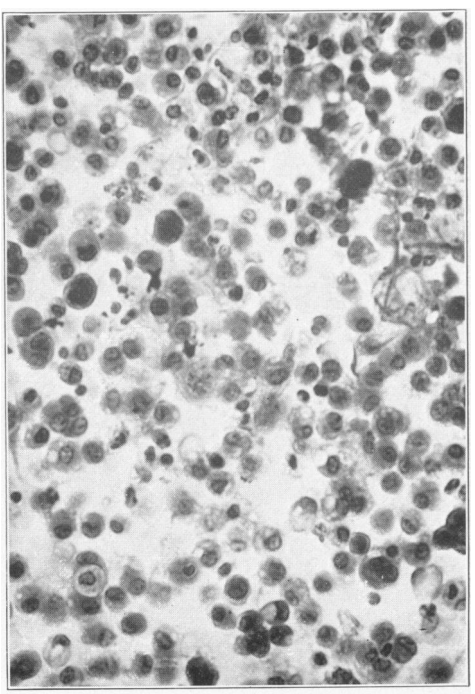

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Foot

Tumor Cells in Sediments of Serous Effusions