The Pathology of Tumors
Part II: Diagnostic Techniques

Juan Rosai, M.D.
Lauren V. Ackerman, M.D.

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Biopsy

Microscopic tissue examination is essential before instituting cancer therapy. Choice of one of the three techniques for obtaining tissue — needle biopsy, incisional biopsy and excisional biopsy — depends upon the location, size and suspected diagnosis of the primary lesion. These techniques, properly performed, carry little risk of infection, hemorrhage or tumor spread in most cases.

Needle Biopsy

There are two different techniques of needle biopsy. In one, tissue is aspirated, smeared on a slide and examined in the same fashion as in exfoliative cytology. In the other, which requires the use of a large bore needle, such as the Vim-Silverman or Menghini model, the tissue obtained is fixed in formalin in toto and processed as

any other biopsy. Aspiration biopsy has been used extensively, and quite successfully, in Scandinavian countries1 and in several hospitals in the United States, particularly at the Memorial Hospital for Cancer and Allied Diseases of New York City. However, we prefer the needle biopsy technique because we feel that the sections obtained are easier to interpret and that they provide important information about the growth pattern of the tumor. Because needle biopsy has some drawbacks as compared with the incisional biopsy (e.g., a smaller amount of tissue is obtained) we recommend a needle biopsy only if the performance of an open biopsy is inconvenient for some reason.

There are situations where needle biopsy is especially useful, for example, in the diagnosis of prostatic carcinoma, where it has become a routine procedure (Fig. 1). Large mediastinal masses are often approached by this technique and needle biopsy is also an excellent method for confirming a diagnosis in prostatic carcinoma, inoperable breast cancer or in peripheral lung cancers with pleural invasion (Figs. 2-4). It is quite successful in detecting metastatic carcinomas to the liver and parametrical extensions of cervical carcinomas. Schajowicz has used it extensively, with excellent results, for the diagnosis of bone tumors2 (Fig. 5). Needle biopsy is very useful for tumors located in the vertebral column, a site otherwise difficult to reach. The technique is also helpful for

Dr. Rosai is Professor, Laboratory Medicine and Pathology and Director of Anatomic Pathology, Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, Minnesota.

Dr. Ackerman is Professor of Pathology, State University of New York, Stonybrook, New York.
the detection of carcinomas metastatic to lymph nodes (Fig. 6). Pathologic confirmation of carcinoma of the head of the pancreas, which is obviously desirable before performing Whipple's operation, can often be made by inserting a needle into the pancreas and examining the material obtained by frozen section.3

For operable tumors of breast, salivary glands and soft tissue, where the microscopic diagnosis is so important in determining the operation to be performed, needle biopsy can be an alternative to incisional or excisional biopsy with frozen section. Although both methods are satisfactory, the occurrence of a few false-negative diagnoses with the needle biopsy have made the frozen section approach more popular.

A cause of concern whenever needle biopsy is contemplated is the possibility of tumor implantation along the needle tract. There is no question that this complication may occur, although the incidence is extremely low. We have seen it with renal cell carcinoma, melanoma, chondrosarcoma and salivary gland carcinoma. To circumvent this problem, the biopsy must be planned so that at operation the surgeon will encompass the zone of the needle tract.

Incisional Biopsy

Incisional biopsy, whether performed with a knife or a punch, is the most common method for making the diagnosis of cancer. For large ulcerated tumors located in the skin or mucous membranes, it is important that the biopsy be taken from the edge, including a small portion of normal skin or mucosa. Biopsies taken from the center of the lesion often show only inflammatory cells or necrotic debris. An exception to this rule is a biopsy taken to rule out the malignant transformation of a villous adenoma of colon. In this case the specimen should be obtained from the center, especially if an indurated area is present. It is also important that the biopsy be deep enough to include a good amount of underlying stroma, because otherwise the invasive nature of the lesion cannot be determined. This is especially true in cases of verrucous or papillary carcinomas, where a superficial biopsy may show only surface epithelium. If a given lesion shows a heterogeneous gross appearance, it is wise to take two or more biopsies from different areas, because sometimes a single biopsy will not show the diagnostic pathologic changes. The piece of tissue obtained should be handled with great care. Pinching a small biopsy with forceps can ruin otherwise perfect material. A "hot knife" should never be used when excising tissues for microscopic examination, because the margins of the lesion or its surface may be so charred or distorted that a microscopic diagnosis may be impossible.

The assumption that incisional biopsy may contribute to the spread of the tumor and therefore decrease the chances of cure has never been proved. Comparison of recurrences and survival rates in tumors excised following an incisional biopsy, as opposed to those removed without previous biopsy, have shown no differences. Following incisional biopsy of a peripheral lung tumor, one consistently finds free cancer cells in the pleural fluid.4 However, these patients do as well as those in whom an incisional biopsy was not performed. Even if a minor risk is involved, the information gained by an incisional biopsy amply justifies the procedure.

Excisional Biopsy

For many tumors of small size, excisional biopsy is preferable to an incisional one, because it can be both of diagnostic and therapeutic value. For example, the majority of pigmented skin lesions will turn out to be benign nevi, seborrheic keratoses or pigmented basal cell carcinomas and will be cured by the procedure. A few will prove to be malignant melanomas which will probably require wide reexcision.

Specimens from excisional biopsies should be removed in one piece, whenever possible. This is extremely important in the evaluation of polyps of colon. As we have already mentioned, the surgical approach to a polyp with carcinomatous change varies a great deal depending on
Fig. 1: Needle biopsy of prostate, showing a poorly differentiated carcinoma replacing part of the specimen.

Fig. 2A and 2B: Roentgenograms of chest showing a large anterior mediastinal mass. It was impossible to determine by x-ray its exact nature. It proved to be a thymoma by needle biopsy.

Fig. 3: Needle biopsy of prostate. Extensive adenocarcinoma is present, exhibiting two different patterns of growth.

Fig. 4: Needle biopsy of pleura demonstrating invasion by a peripherally located lung carcinoma.

Fig. 5: Needle biopsy of bone lesion located in the lower femoral end in a 16-year-old female. The presence of regularly distributed osteoclast-like cells in a cellular stroma are diagnostic of giant cell tumor.

Fig. 6: Needle biopsy of inguinal lymph node showing metastatic carcinoma accompanied by abundant desmoplastic reaction. The primary tumor was a papillary serous cystadenocarcinoma of the ovary.
the presence or absence of cancer at the base of the polyp. It is obvious that this would be impossible to evaluate if the polyp has been removed in pieces. Every effort should be made by the pathologist to determine the adequacy of the excision. For this purpose, sections are carefully taken from the lateral and deep margins (Fig. 7). To be completely sure that one is looking at the true surgical margin when examining the microscopic slides, we have found it useful to paint the margins with diluted India ink before any sections are taken. India ink remains after formalin fixation and paraffin embedding, and can be identified as a thin black line at the edge of the section (Fig. 8). If the excision is inadequate, the pathologist should indicate the point where the surgeon has apparently cut through tumor. The surgeon can then use this information either for further immediate surgery, or as a guide in subsequent follow-up.

**Diagnostic Cytology**

The value of this procedure has increased to such a degree that diagnostic cytology is now rightfully regarded as a subspecialty of pathology in its own right. From the timid terminology employed only a few years ago ("suspicious," "Grade III," etc.), the accuracy of the procedure in experienced hands is such that a diagnosis of cancer on cytologic grounds carries the same weight as that of a tissue biopsy.

It is now common practice to write the cytology reports using the same language as for the microscopic sections, instead of employing the original grading system of Papanicolaou. A surgeon has a clearer idea of the nature of a pulmonary mass if the cytologic report of sputum is "squamous cell carcinoma" instead of "cytology grade IV." It should be realized that the value of cytology and the indications for its use vary a great deal from organ to organ. In sites where a biopsy can be easily obtained, such as skin, oral cavity or vulva, there is no point in doing cytology. On the other hand, since only a third of pulmonary tumors can be diagnosed by bronchoscopy, the detection of cancer cells in sputum or bronchial washings is often the only way to morphologically document a lung cancer before surgery or radiation therapy is instituted. Three sputum specimens, taken on consecutive days, are recommended as the routine procedure. Sputum expectorated immediately after a bronchoscopy may sometimes...
show malignant cells when the other specimens have been negative. The diagnostic accuracy of lung cytology is high. If the specimens have been taken as indicated, about 80 percent of the carcinomas will be detected. The rate of detection used to be much higher (80 percent or more) for squamous cell carcinoma and oat cell carcinoma than for adenocarcinoma (50 percent), due to the often peripheral location of the latter. With the introduction of the fiberoptoscope, which can reach very small and peripheral branches of the bronchial tree, this is no longer the case (Figs. 9-11). In addition to making the diagnosis of cancer, the pathologist can in most instances classify the exact type of tumor from the cytologic findings alone.

Cytology is also of great importance in the diagnosis and follow-up of endometrial and cervical lesions, especially the latter. A cervical smear is taken of every adult woman who is admitted to our institutions, whatever the reason for her admission. Screenings of large segments of the population have been carried out with excellent results in Memphis, Vancouver and other cities. If it were possible to screen the entire female population at appropriate time intervals, carcinoma of the cervix as a cause of death might be completely eliminated.

The cervical smear detects more than 95 percent of the cases of dysplasias and carcinoma in situ, and about 90 percent of the invasive carcinomas. Invasive carcinomas often undergo secondary changes, such as ulceration, with the resulting exfoliation of red blood cells, inflammatory cells and detritus, which tend to obscure the diagnostic features. It is fortunate that cytology is more often positive with dysplasias and in situ carcinomas than with the invasive tumors, because most invasive carcinomas will be detected clinically, whereas the former conditions are often unrecognizable to the naked eye. Cytology of a cervical smear will detect more dysplasias and in situ carcinomas than a random cervical biopsy; however, if a suspicious area is clinically apparent, a biopsy of this area should be taken in addition to obtaining smears for cytology. In contrast to lung cancer, we do not feel that definitive treatment of a uterine tumor should be instituted on the basis of a positive cytologic finding alone, because of the accessibility of the cervix and endometrium to cone biopsy and curettage, and the unlikely possibility of spread by these procedures.

The terminology we have adopted for reporting cervical cytology is the same as that used for cervical biopsies. We use dysplasia (which is graded as mild, moderate or severe), carcinoma in situ and invasive carcinoma (Figs. 12-14). This system is helpful to the gynecologist, because it allows him to manage the patient accordingly. If the cytologic diagnosis is that of mild or moderate dysplasia (and assuming that the cervix shows no obvious lesions), he can safely follow this patient with a repeat cytology at a six month or one year interval. If the cytologic diagnosis is that of severe dysplasia, carcinoma in situ, or invasive carcinoma, a cervical biopsy (preferably under colposcopic guidance) is indicated. Cervical cytology is also helpful in detecting recurrences in cancer patients treated by either surgery or radiation therapy.

The rate of detection of endometrial carcinoma is not as good as for the cervical lesions. About 65 percent of them are diagnosed or suspected by the cytologic findings. Specimens taken from the vaginal "pool" are most useful, because both normal and neoplastic endometrial cells accumulate in this area after they are exfoliated.

Over 70 percent of gastric carcinomas can be detected by cytology. The method is of greater value for radiographically debatable lesions, and in cases where a definitive diagnosis would modify the therapy (Fig. 15). Some ulcerated esophageal
cancers, difficult to biopsy because of the surrounding edema, can sometimes be diagnosed by a direct smear of the lesion.

Cytology of urine is of practical value in the detection of transitional cell carcinomas, but not for renal cell carcinomas which do not exfoliate into the urine until the pelvis has been invaded (Fig. 16). In transitional cell carcinomas, Grade 1 tumors are often missed because they are extremely well differentiated. The method is obviously of greater value for the detection of pelvic and ureteral tumors than it is for bladder neoplasms, because the latter can be readily visualized and biopsied, except in the rare instance when they are located inside a diverticulum.

Cytologic specimens of pleural and peritoneal effusions are probably the most challenging to interpret, because of the bizarre appearance of some hyperplastic mesothelial cells.

Needle, incisional and excisional biopsies, used appropriately, are capable of safely obtaining tissue for microscopic examination from most types of lesions. However, in cases where histologic diagnosis is impossible prior to surgery or radiotherapy, or for detection of asymptomatic cancer of some sites, exfoliative cytology has become an excellent indicator of malignancy, particularly in cervical or endometrial cancer.

We have found that terminology employed in reporting biopsy results is also useful in cytology reports and can give the clinician a clearer idea of the pathologist's findings than the grading system originally used by Papanicolaou.

**Frozen Section**

Frozen section diagnosis has proved of great value in helping the surgeon determine the proper course of action while the patient is still in the operating room. This method of diagnosis originated in the United States where it is used more frequently than in any other country.

There are two major indications for the performance of frozen sections: (1) to make a therapeutic decision on the basis of the diagnosis rendered; and (2) to be sure that diagnostic tissue has been obtained in cases where the purpose of surgery is to obtain a biopsy, such as in a laparotomy done to confirm a suspected diagnosis of lymphoma.

The responsibility for frozen section diagnosis should be in the hands of an expert pathologist, with conservative attitudes and good judgment. Before rendering a diagnosis, the pathologist should have all the clinical data pertaining to the case. If the patient has had a previous biopsy or operation, the pathologist should obtain and review the slides in advance so a comparison can be made with the material obtained at frozen section. If the pathologist goes to the operating room he can observe the lesion in situ, learn about the surgical findings and sometimes make a suggestion about where to take the biopsy. Although the decision about the therapy is obviously made by the surgeon, a discussion with the pathologist about the natural history of the lesion (such as tendency of recurrence according to the procedure, incidence of bilaterality and so on) can be helpful to the surgeon. The pathologist's main responsibility, however, is to give an accurate microscopic diagnosis. The surgeon must have complete confidence that the pathologist will be conservative, always conscious of the patient's welfare, and, that if the pathologist diagnoses cancer, he will be correct in the overwhelming majority of cases.

Some refinements in the technique have increased the speed of the method and improved the quality of frozen sections. The use of the cryostat has been a great improvement over the CO₂ microtome. The sections are thinner and easier to obtain and serial sections from different levels of the block can be taken whenever required. Multiple small fragments of tissue (for example, tissue obtained from
The Permount, men.

The value of a positive frozen section diagnosis must always be weighed against the risk of implantation. In lesions of the pancreas, for instance, which involve extensive surgery associated with high mortality, high morbidity and low curability rates, a frozen section should always be obtained. Conversely, if a lesion of the cecum, diagnosed radiographically as cancer, is in doubt at operation, ileocolicectomy should be done without frozen section. In this case, the risk of implantation is too high to justify biopsy after opening the colon, whereas in our institution ileocolicectomy carries practically no mortality or morbidity.

We have found that frozen sections may be conveniently and accurately done on needle biopsies; this technique has been especially useful in biopsies of the pancreas through the duodenum, and of the prostate through the rectum or perineum.

The area most commonly examined by frozen section method is the breast. The treatment performed, depending on the result of the microscopic examination and the bias of the surgeon, is an excisional biopsy, simple mastectomy, "total mastectomy" or the traditional radical mastectomy. Some rare types of breast cancers which practically never metastasize to the regional lymph nodes, such as the pure mucinous (Fig. 18) and the adenoid cystic types, are adequately treated by simple mastectomy.\(^7,8\) The relative value of simple mastectomy, total mastectomy and radical mastectomy as well as the role of radiation therapy and chemotherapy for this disease are controversial. Several randomized studies are now in progress to resolve some of these issues. In cases of lobular carcinoma, a tumor with a high tendency for bilateral involvement, a generous blind biopsy of the upper lateral quadrant of the other breast is recommended by some,\(^9\) although its ultimate value remains to be proven.

Fig. 9: Cytologic specimen of sputum, containing cells of epidermoid carcinoma of the lung. Note the bizarre nuclear configuration, abnormal chromatin distribution and keratinized cytoplasm.

Fig. 10: Cells of adenocarcinoma of lung. A large nucleolus is present in most of the cells. The cytoplasm is vacuolated.

curettage of the uterine cavity) can be cut simultaneously by using a synthetic adhesive which holds all the fragments together in a single block, somewhat resembling the action of paraffin in the routine embedding procedure. To make the procedure as fast as possible, we freeze the block of tissue in isopentane cooled by either a mixture of dry ice and acetone or by an electronic device, which results in almost instantaneous freezing of the specimen. The sections are stained with hematoxylineosin, dehydrated and mounted in Permount, using the same sequence as for the paraffin-embedded material (Fig. 17). With few exceptions, special stains have no practical applications for frozen section diagnosis.

Removing tissue for frozen section carries the danger of tumor implantation. Consequently, the surgeon should plan his procedure so that if the lesion proves to be cancer, the entire area of biopsy can be excised with the lesion. The value of a positive frozen section diagnosis must always be weighed against the risk of implantation. In lesions of the pancreas, for instance, which involve extensive surgery associated with high mortality, high morbidity and low curability rates, a frozen section should always be obtained. Conversely, if a lesion of the cecum, diagnosed radiographically as cancer, is in doubt at operation, ileocolicectomy should be done without frozen section. In this case, the risk of implantation is too high to justify biopsy after opening the colon, whereas in our institutions ileocolicectomy carries practically no mortality or morbidity.

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Fig. 11: Cells of oat cell carcinoma of lung. The size is small, only slightly larger than that of a lymphocyte. Nuclear aberrations are evident. The cytoplasm is extremely scanty.

Fig. 12: Moderate dysplasia of uterine cervix. The nuclei are enlarged, with abnormal chromatin distribution. A good amount of cytoplasm is present.

Fig. 13: Carcinoma in situ of cervix. This is a cluster of so-called "third cells."

Fig. 14: Invasive epidermoid carcinoma of cervix. There is more pleomorphism than with the in situ lesions.

Fig. 15: Cells of gastric adenocarcinoma. A lesion had been seen radiographically, but the diagnosis was unclear.

Fig. 16: Cells of transitional cell carcinoma of bladder.
In cases of unilateral ovarian tumors, the choice between unilateral salpingo-oophorectomy and bilateral salpingo-oophorectomy with total hysterectomy largely depends on the microscopic type of ovarian neoplasm.

Nodules in the thyroid and salivary gland should always be taken with a margin of normal tissue (which in thyroid nodules implies lobectomy) and examined by frozen section. Adenoid cystic carcinomas of salivary gland often clinically simulate a benign tumor; however, despite their slow growth rate, they will prove fatal in practically every case, unless completely excised the first time they are encountered. Since this tumor is well known to invade beyond its gross margin, frozen section is the only way to recognize it and insure that all the tumor has been excised (Fig. 19).

There is still a generally held opinion that frozen sections are unreliable for tumors of the thyroid gland. This false impression is partially based on the diagnostic problem presented by the so-called "adenomas with malignant change," "angioinvasive adenomas" or low-grade follicular carcinomas. These tumors look cytologically like benign follicular nodules, but in some areas they show evidence of either capsular or vascular invasion. This feature can be missed in a frozen section. But this does not create a therapeutic problem; the treatment is the same as for the benign thyroid gland nodule, that is, simple lobectomy. Other thyroid lesions rarely present a diagnostic problem. There is usually no difficulty in recognizing an adenomatous nodule, a focus of thyroiditis or a papillary, undifferentiated or infiltrative follicular carcinoma.

The record of the 2,240 consecutive frozen sections done at Barnes Hospital was reviewed. The results are detailed in Table 1. There were 13 false-negatives and only five false-positives, resulting in an overall accuracy of 99.3 percent, and a false-positive incidence of 0.2 percent. Reports from other institutions, including a recent review of the experience at the University of Minnesota Hospitals, show very similar results.

**GROSS AND MICROSCOPIC EXAMINATION**

Autopsy material, of course, yields a great deal of useful information as to the effects of surgical or radiotherapeutic treatment as well as of the dissemination of the neoplasm and its secondary effects. However, we are concerned mainly with the description of surgical specimens.

Cancers growing in different organs have different patterns depending on their size, cellular components, stroma and location. In the cecum, a cancer may form a bulky mass because of the large space available; in the breast, the lesion is frequently hard because of dense stroma associated with the cancer.

Polyps of the cecum are usually sessile whereas those of the sigmoid are commonly pedunculated, probably because of the different consistency of the feces in these two areas, with the resulting different pulling effect on the bowel mucosa.

The experienced pathologist learns to recognize certain classic patterns for the cancers in various organs. The bright yellow color of a cancer of the kidney, the exophytic lesion of an advanced cancer of the uterine cervix, the retracted pleura in a peripheral carcinoma of the lung; these lesions all result in definite diagnostic patterns (Figs. 20-22). Some tumors, however, have a bizarre or non-specific gross appearance and cause difficulty in gross recognition.

We believe that storing gross material indefinitely is unnecessary if the specimens are carefully photographed, examined and multiple sections taken.
Instant (Polaroid®) photographs of the gross specimens are especially useful. They can be attached without delay to the pathology report, and can also be used for marking the exact area where various blocks have been taken from large specimens (Fig. 23). This is a more accurate way of recording the study of a specimen than relying on a pathologist's drawing.

Roentgenograms of the surgical specimens taken with a small portable x-ray machine and instantly developed are also very helpful. In addition to adding useful information to the study of bone lesions (Fig. 24), calcified tumors and intraluminal lesions (the latter after the injection of opaque material), this method is of diagnostic importance in cases of asymptomatic breast carcinomas diagnosed by mammography. A positive x-ray of the surgical specimen confirms to the surgeon that the lesion in question has been excised, and greatly facilitates its gross detection by the pathologists, a task otherwise difficult because of the extremely small size of many of these tumors.14

Tumors of some organs, such as lung, bowel, kidney and specimens of pelvic exenteration, are better examined by the injection of formalin into their natural cavities and blood vessels, followed by immersion of the entire organ in formalin before it is sectioned (Fig. 25). In this way the in vivo appearance and relationship of the tumor are preserved and correlation between the gross specimen and the radiologic findings is achieved.

In sectioning some specimens, we now use an electrically driven commercial meat slicer, which produces slices of uniform thickness virtually free of knife marks. In addition to cutting very uniform sections for gross demonstrations and photographs, this instrument can serially section a specimen in slices one mm thick, for thorough gross examination. This is especially indicated for seemingly solitary metastases in lobectomy specimens, or in apparently normal spleens removed for the staging of Hodgkin's disease.

At the present time, cancer surgery varies from small skin excisions to pelvic exenteration and hemipelvectomy. The pathologist is responsible for proper orientation of the specimen and careful description of the tumor, but there are other responsibilities that reflect on the prognosis and further treatment of the patient. For instance, it is not sufficient to describe the color, consistency and size of a breast cancer. The local extent and the general type of the tumor should be carefully investigated and charted. Specimens may have critical areas that are close to the limits of the wound, and it is the responsibility of the surgeon and the pathologist to confer concerning these areas and to take carefully oriented sections in order to prove or disprove the adequacy of the excision.

When groups of lymph nodes have been dissected, the pathologist must meticulously orient these groups, place them in separate containers, and examine them separately in order to determine the extent of involvement. With thorough examination, the pathologist will be able to report how many nodes and which nodal groups are involved. We do not believe that clearing of lymph node dissections or serial sectioning as a routine procedure is of practical value. Using this method, Pickren found occult metastases in 22 percent of 51 cases in which the axillary lymph nodes had been classed as negative by routine sectioning.15 However, this finding had no practical value from the standpoint of prognosis because the survival rate of these patients was the same as that of patients without lymph node involvement. The average number of nodes in our radical mastectomy specimens is 29, neck dissections 49, and abdominoperineal resections 30. Diagrams are attached to written reports which help the clinician summarize the extent of the neoplasms. These charts have been made for neck dissection.
radical mastectomy, pelvic exenteration, laryngectomy, abdominoperineal resection, pneumonectomy and numerous other operations (Fig. 26).

The microscopic description of tumors includes a detailed account of the microscopic pattern, nuclear changes, stroma, etc. Some of these changes are important to the pathologist, but not necessarily to the clinician. Only those microscopic findings that have prognostic or therapeutic significance should be presented. For example, certain breast tumors (lesions with a papillary pattern, mucinous carcinoma and medullary carcinoma with lymphoid stroma) carry a much better prognosis than many other histologic patterns of breast cancer. Interstitial cell hyperplasia associated with a malignant testicular tumor indicates a less favorable outcome (Fig. 27).

The relationship of a carcinoma to the adjacent host tissue may also be clinically significant. A well circumscribed carcinoma that seems to push against the adjacent tissues, with degenerative changes and a lymphocytic infiltrate in this area, may imply longer survival than a tumor that invades adjacent tissues (Figs. 28 and 29).

The importance of this finding has been demonstrated in lesions of the stomach, breast and large bowel. The proportion of pushing types is relatively high in carcinoma of the large bowel and low in carcinoma of the stomach. However, a pushing border in carcinoma of the stomach is also significant. In a series of 180 patients with gastric carcinoma reported from Barnes Hospital there were 25 five-year survivors. Of the 45 cases with a pushing border, 11 (25 percent) survived. An additional seven of the 25 survivors had carcinomas with a mixed pushing border and some areas of infiltration. This finding may represent some sort of biological interaction between the patient and his cancer, for these patients survive longer even when lymph node metastases are present.

With mastectomy specimens we try to summarize the pertinent pathologic findings in a final paragraph, such as in the following example: “The tumor measured
2.5 cm, was sharply circumscribed, well-differentiated, arose in an outer quadrant, and none of the 35 lymph nodes found in the axillary area was positive. These findings are all favorable and the prognosis for this patient should be excellent. We have not as yet seen mediastinal involvement under these circumstances.” Or we might say: “The tumor was large, undifferentiated and had evidence of dermal lymphatic involvement. A mediastinal node, as well as 16 out of the 35 axillary lymph nodes, were implicated by cancer. These findings indicate an extremely poor prognosis.”

Statements of this nature translate a routine pathologic report into constructive information which can give the surgeon a better idea of the prognosis and a firmer basis for further treatment.

Ancillary Studies

Although the majority of neoplasms can be diagnosed by examining hematoxylin-eosin stained sections of paraffin-embedded material, there are many instances when valuable information about the tumor type, histogenesis and functional activity can be obtained by the use of special procedures. It is important to keep this in mind at the time the fresh tissue is received, because some of these techniques cannot be done on formalin-fixed material.

The most common ancillary technique is the use of special stains. In many instances their use can solve a difficult problem in differential diagnosis. A positive mucin stain will confirm the epithelial nature of an undifferentiated neoplasm. The presence of abundant glycogen in the cells of an undifferentiated round cell tumor of bone in a child supports the diagnosis of Ewing's sarcoma (Fig. 30) rather than that of reticulum cell sarcoma or metastatic neuroblastoma.18 Mallory's phosphotungstic acid-hematoxylin or a Masson's trichrome can confirm a diagnosis of rhabdomyosarcoma by demonstrating cross striations in the tumor cells. Reticulum stains are helpful for the classification of vascular tumors.

Immunocytochemical techniques have a degree of specificity and sensitivity which...
Fig. 23: Instant (Polaroid) gross photograph of femur with osteosarcoma. The marks indicate where the sections have been taken. This allows a quick orientation when looking at the microscopic slides and permits a better correlation with the gross findings.

Fig. 24: Roentgenogram of a slice of femur containing an extensive osteosarcoma. The tumor involves the medullary canal, elevates the periosteum (left arrows) and forms a large soft tissue mass. Codman's angle can be clearly seen (right arrow).

can hardly be matched by any other method in pathology. Until recently, their application to the study of human tumors was limited because of the many limitations of the existing methods (mainly based on immunofluorescence), such as the need to perform the reaction in fresh specimens. A revolution in the field has taken place with the introduction by Sternberger of a nonlabeled antibody technique using peroxidase-antiperoxidase complex as a marker. This method can be applied to routinely-processed formalin-fixed and paraffin-embedded material and, therefore, it can be used in retrospective studies. Theoretically, it can demonstrate any antigen in tissues as long as some antigenic determinants have been preserved following the processing and a specific antibody to that antigen is available. The method has already been applied successfully for the demonstration of immunoglobulin, gastrointestinal, pancreatic, pituitary and thyroid hormones, gonadotropin, oncofetal antigens, enzymes and viral antigens. It is of great value for the precise classification of tumors (for instance, the identification of an islet cell tumor as composed of beta, alpha or delta cells on the basis of the presence of insulin, glucagon and somatostain, respectively) and in the differentiation of hyperplastic from neoplastic processes in the lymphoreticular system (by demonstrating a polyclonal proliferation in the first case and monoclonal proliferation in the second, as determined by the presence of one or more immunoglobulin subclasses) (Figs. 31 and 32).

Examination of tumors with the electron microscope may sometimes solve a diagnostic problem. We have found this
technique useful even with formalin-fixed material. The electron microscope can distinguish poorly differentiated carcinomas from malignant lymphomas if tonofilaments and complex desmosomes are found (Figs. 33A and 33B); poorly differentiated myeloma cells can be identified by their numerous flat cisternae of granular endoplasmic reticulum; smooth muscle tumors can be separated from the fibroblastic and Schwannian neoplasms; carcinoid tumors and several other endocrine neoplasms can be easily identified (although not differentiated from each other) by the presence of "neurosecretory" granules in their cytoplasm (Figs. 34A and 34B).

Tissue culture has been used extensively for diagnostic purposes by the pathologists of the Columbia University-Presbyterian Hospital in New York City, apparently with good results. This is especially useful in cases of neuroblastoma where, if the tumor cells grow neurites in 24 hours, the neuronal nature of the neoplasm is confirmed.

Finally, biochemical studies are of great value in the classification of endocrine tumors and, in general, of any tumor with secretory activity. For this purpose, part of the fresh tumor is frozen in isopentane cooled with liquid nitrogen, and is kept in a deep freezer until the desired biochemical determinations can be performed.

Summary

Frozen section diagnosis of cancer is generally used to 1) determine the type and extent of treatment while the patient is in the operating room and 2) to confirm the adequacy of surgical excision.
Frozen section diagnosis forms the basis for further surgery. Because of the pathologist's role in this decision, he should be as familiar as possible with pertinent clinical data (e.g., previous biopsy or surgery) prior to operation. In some cases he may wish to observe the lesion in situ or suggest an area to be biopsied.

Frozen section is very reliable in cancer diagnosis. A recent review of 2,240 consecutive sections has shown an overall accuracy of 99.3 percent with 13 false-negatives and only five false-positives. The technique is useful for most specimens, even tissue from needle biopsies.

When the pathologist receives the gross specimen he must orient it and describe the color, consistency and size of the tumor and record its appearance prior to sectioning. The specimen must be carefully examined for any indications of inadequate tumor excision. Specimens may have critical areas close to the wound limits and the sections must be carefully oriented to prove or disprove adequate excision. Specimen roentgenography is very useful in evaluating excision and in pinpointing the tumor area.
Fig. 27: Hyperplasia of Leydig's cells in the normal testis adjacent to a malignant testicular tumor. This finding is associated with a decreased survival rate.

Fig. 28: Medullary carcinoma of breast. The border of the tumor is "pushing." This feature is associated with a good prognosis.

Fig. 29: Same tumor, showing numerous lymphocytes among the tumor cells. This may represent an attempt of resistance by the host and is also indicative of good prognosis.

Fig. 30: PAS stain in Ewing's sarcoma of bone. The cytoplasm of the tumor cells contains PAS-positive granules. All of this material disappeared following diastase digestion, indicating that it was composed of glycogen.

Fig. 31: Immunoglobulin of the IgA type in the cytoplasm of plasma cells is selectively demonstrated with the unlabeled peroxidase method of Sternberger.

Fig. 32: Human chorionic gonadotropin is shown in the cytoplasm of multinucleated giant cells present in an otherwise typical seminoma of the testicle. This feature is associated with a decreased survival. The technique used was an immunoperoxidase method directed against the beta subunit of the hormone.
Microscopic description of tumors includes details of pattern, nuclear changes, stroma, and many others. However, only the microscopic findings which have prognostic or therapeutic significance should be emphasized to the surgeon. Summarizing the pertinent findings in a final paragraph of the pathology report can translate routine microscopic description into constructive clinical information which will give the surgeon a clear idea of the probable behavior of the tumor.

In addition to the standard procedures for preparing and examining specimens for final diagnosis, there are some special techniques which are extremely useful. These include immunohistochemical staining, electron microscopy, tissue culture and biochemical studies.

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Fig. 33B: Electron micrograph of the same lymph node, prepared from formalin-fixed material. The presence of tonofilaments (T) and complex desmosomes (arrow) indicated that it was metastatic carcinoma. The primary tumor was found in the nasopharynx.

Fig. 34A: Light microscopy of a small nodule found in an appendix which was removed incidentally. A glandular neoplasm infiltrates the muscular wall. The diagnoses of primary and metastatic adenocarcinoma were considered.

Fig. 34B: Electron micrograph of the same specimen. The tumor cells contain a large number of secretory granules composed of a central dark core and an envelope, with a clear halo in between. This finding indicated that we were dealing with an atypical carcinoid tumor.

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