The Pathology of Tumors, Part Three

Frozen Section
Gross and Microscopic Examination
Ancillary Studies

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

**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 his 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 curettage of the uterine cavity) can be cut simultaneously by using a synthetic adhesive which holds all the fragments together in a

---

*Dr. Ackerman is Professor of Surgical Pathology and Pathology, Washington University School of Medicine, and Surgical Pathologist, Barnes Hospital and affiliated hospitals, St. Louis, Missouri.*

*Dr. Rosai is Assistant Professor of Pathology, Washington University School of Medicine, and Surgical Pathologist, Barnes Hospital and affiliated hospitals.*

270
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 hematoxylin-eosin, dehydrated and mounted in Permount, using the same sequence as for the paraffin-embedded material. (Fig. 1.) In general, special stains have no practical applications for frozen section diagnosis, with the exception of stains for mucin, which we have found helpful in the diagnosis of signet cell-type mucinous carcinomas. The stain we use for this purpose is Alcian blue at pH 2.5 for 1 or 2 minutes.

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, ileoectomy 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 ileoectomy 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 the frozen section method is the breast. The treatment performed, depending on the result of the microscopic examination, is usually excisional biopsy or radical mastectomy. Some rare types of breast cancers which practically never metastasize to the regional lymph nodes, such as the pure mucinous (Fig. 2) and the adenoid cystic types, are adequately treated
by simple mastectomy. 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 can be taken during the same operation.

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. 3.)

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 readily missed in a frozen section. However, this does not create a therapeutic problem, because 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.

A relatively new application of frozen section has been found in the management of patients in whom a biopsy of the uterine cervix has shown either severe dysplasia or carcinoma in situ. A conization is performed, and the specimen is examined in toto by the frozen section technique. If all of the lesion is either dysplasia or carcinoma in situ, the surgeon may stop the operation there or perform an hysterectomy, depending on the pathology. If stromal invasion is present, intracavitary radium can be implanted during the same operation. The method is accurate and benefits the patient. However, this type of examination requires far more time and effort than the ordinary frozen section. One pathologist and three technicians, working with two cryostats, is the ideal team needed in order to examine the whole specimen in a reasonably short time.

The record of the 2,240 consecutive frozen sections done at Barnes Hospital was recently reviewed. The results are detailed in the Table on page 280. There were 13 false-negatives and only 5
false-positives, resulting in an overall accuracy of 99.3 percent, and a false-positive incidence of 0.2 percent. Similar results have been reported from other institutions.7

**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 cecum are usually sessile whereas those of sigmoid polyp 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 of a cancer of the kidney, the ulcerating lesion of an advanced cancer of the larynx, the cancer in the lung with the retracted pleura; these lesions all form definite diagnostic patterns. (Figs. 4-6.) Some tumors, however, have a bizarre pattern and cause difficulty in gross recognition.
We believe that storing gross material is unnecessary if the specimens are carefully photographed, examined and multiple sections taken. A high percentage of these specimens can then be discarded and the paraffin blocks kept indefinitely.

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. 7.) 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. 8), 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.

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. 9.)

---

Fig. 7. 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. 8. 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).
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. Breast specimens may be submitted in two portions with only a small sliver of skin, a variable amount of pectoral muscle and a few axillary nodes. Such gross material is an indication that the surgeon is not familiar with what is called an adequate radical mastectomy. Specimens may have critical areas which 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 bottles, 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. 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

In this way the in vivo appearance and relationship of the tumor are simulated 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 perfect sections for gross demonstrations and photographs, this instrument can serially section a specimen in slices 1 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

Fig. 9. Sagittal section of a specimen of total pelvic evisceration, obtained after fixation of the bladder, uterus and large bowel by injection of formalin. The topography of the lesion can be clearly demonstrated. This is a case of postirradiation persistence of epidermoid carcinoma of cervix. The tumor is also involving the posterior vagina and the rectovaginal septum.
made for neck dissection, radical mastectomy, pelvic exenteration, laryngectomy, abdominoperineal resection, pneumonectomy and numerous other operations. (Fig. 10.)

The microscopic description of tumors includes a detailed account of the microscopic pattern, nuclear changes, stroma, etc. These changes are important to the pathologist, but not necessarily to the clinician. However, those microscopic findings which 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.\(^\text{10}\) Interstitial cell hyperplasia associated with a malignant testicular tumor suggests a less favorable outcome. (Fig. 11.)

The relationship of a carcinoma to the adjacent host tissue may also be clinically significant. A well circumscribed carcinoma which seems to push against the adjacent tissues, with degenerative changes and a lymphocytic infiltrate in this area, may imply longer survival than a tumor which invades adjacent tissue. (Figs. 12 and 13.) The importance of this finding has been demonstrated in lesions of the stomach\(^\text{11}\), larynx\(^\text{12}\), 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 significant. In a series of 180 patients with gastric carcinoma reported from this institution there were 25 five-year-survivors. Of the 45 cases with a pushing border, 11 (25 percent) survived. An additional 7 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 breast specimens we try to summarize the pertinent pathologic findings in a final paragraph: “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 were 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, 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 will indicate that it is a Ewing’s sarcoma (Fig 14) rather than a reticulum cell sarcoma or a metastatic neuroblastoma. 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.

Immunofluorescent stains have a degree of specificity in pathology which can hardly be matched by any other histochemical technique. Their use in the study of neoplasms has been limited, but there is every indication that they will become an important diagnostic tool. Immunofluorescent staining has been used successfully in the identification of actomyosin in smooth and skeletal muscle tumors, gonadotropin in functioning bronchogenic carcinomas, and immunoglobulins in plasma cell myeloma and Waldenström’s macroglobulinemia.

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 tonofibrils and complex desmosomes are found (Figs. 15A and 15B); poorly differentiated myeloma cells can be identified by their numerous flat cisternae of granular endoplasmic reticulum; smooth muscle tumors can be separated from 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. 16A and 16B.)

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.
Fig. 16A. 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. 15B. 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. 16B. 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.
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 over-all accuracy of 99.3 percent with 13 false-negatives and only 5 false-positives.

---

**FROZEN SECTION DIAGNOSIS IN 2,240 CONSECUTIVE CASES**

Barnes Hospital
Washington University School of Medicine
St. Louis, Missouri

| Organ                  | No. of Cases | Benign Lesions | Malignant Lesions | False Positives | False Negatives | Diagnosis Deferred |
|------------------------|--------------|----------------|-------------------|-----------------|-----------------|--------------------|
| Breast                 | 679          | 437            | 202               | 0               | 3 (0.4%)        | 6 (0.9%)           |
| Soft tissues           | 298          | 135            | 163               | 1 (0.3%)        | 1 (0.3%)        | 7 (2.3%)           |
| Gastrointestinal tract | 251          | 192            | 59                | 0               | 3 (1.1%)        | 6 (2.5%)           |
| Lymph nodes            | 232          | 108            | 124               | 0               | 1 (0.4%)        | 0                  |
| Lung                   | 169          | 49             | 120               | 2 (1.1%)        | 0               | 0                  |
| Thyroid                | 112          | 100            | 12                | 0               | 0               | 5 (4.4%)           |
| Central nervous system | 112          | 18             | 94                | 1 (0.8%)        | 2 (1.7%)        | 4 (3.5%)           |
| Bone and joints        | 79           | 42             | 37                | 0               | 1 (1.2%)        | 5 (6.3%)           |
| Liver and gallbladder  | 73           | 29             | 44                | 0               | 0               | 1 (1.3%)           |
| Pancreas and bile ducts| 45           | 22             | 23                | 0               | 2 (4.4%)        | 0                  |
| Parathyroid            | 44           | 44             | 0                 | 0               | 0               | 0                  |
| Skin                   | 51           | 18             | 33                | 0               | 0               | 0                  |
| Miscellaneous          | 135          | 73             | 62                | 1 (0.7%)        | 0               | 4 (2.9%)           |
| **TOTAL**              | **2,240**    | **1,267**      | **973**           | **5 (0.2%)**    | **13 (0.5%)**   | **38 (1.6%)**      |

*E.N.T. and gynecologic cases excluded.*
tives. 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.

Microscopic description of tumors includes details of pattern, nuclear changes, stroma, etc. The microscopic findings which have prognostic or therapeutic significance are reported to the surgeon. However, some features which are important to the pathologist may have no value to the clinician. 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 immunofluorescence staining, electron microscopy, tissue culture and biochemical studies.

References

1. Norris, H. J., and Taylor, H. B.: Prognosis of mucinous (gelatinous) carcinoma of the breast. Cancer 18: 879-885, 1965.
2. Cavens, F. J., and Taylor, H. B.: Adenoid cystic carcinoma of the breast. An analysis of 81 cases. Cancer 24: 740-745, 1969.
3. Neuman, W.: Lobular carcinoma of the female breast. Report of 72 cases. Ann. Surg. 164: 305-314, 1966.
4. Smith, L. C.; Lane, N., and Rankow, R. M.: Cylindroma (adenoid cystic carcinoma): A report of fifty-eight cases. Amer. J. Surg. 110: 519-526, 1965.
5. Kaufman, R. H.; Janes, O. G., and Cox, H. A.: Cervical conization with frozen section diagnosis. Amer. J. Obstet. Gynec. 92: 71-77, 1965.
6. Elsner, B.: La biopsia por congélación: su valor asistencial y en la educación médica de los patólogos. Prens Med. Argent. 55: 1741-1749, 1968.
7. Nakasawa, H.; Rosen, P.; Lane, N., and Latten, R.: Frozen section experience in 8000 cases. Accuracy, limitations, and value in residency training. Amer. J. Clin. Path. 49: 41-51, 1968.
8. Koehl, R. H.; Snyder, R. E.; Hutter, R. V. P., and Foote, F. W.: The incidence and significance of calcifications within operative breast specimens. Amer. J. Clin. Path. 53: 3-14, 1970.
9. Pickren, J. W.: Significance of occult metastases. A study of breast cancer. Cancer 12: 1266-1271, 1961.
10. Dehner, L. P., and Taylor, H. B.: Medullary carcinoma of the breast. A clinicopathologic study of 39 cases. Cancer (in press).
11. Monofo, W. W.; Krause, G. L., and Medina, J. G.: Carcinoma of the stomach. Morphological characteristics affecting survival. Arch. Surg. (Chicago) 80: 754-763, 1962.
12. McGovran, M. H.; Bauer, W. C., and Ogura, J. H.: The incidence of cervical lymph node metastases from epidermoid carcinoma of the larynx and their relationship to certain characteristics of the primary tumor. A study based on the clinical and pathological findings for 96 patients treated by primary en block laryngectomy and radical neck dissection. Cancer 11: 55-66, 1961.
13. Schajowicz, F.; Ewing's sarcoma and reticulosarcoma of bone; with special reference to the histochecmical demonstration of glycogen an aid to differential diagnosis. J. Bone Joint Surg. 41-A (3): 249-356, 1959.
14. Kao, V. C. Y.; Goff, P. W., and Rappaport, H.: Leiomyoma of the uterus. A histologically problematic rare tumor confirmed by immunohistochemical studies. Cancer 21: 585-598, 1968.
15. Himamoto, R.; Jurandowski, J.; Bernecky, J., and Presman, D.: Immunohistochemical differentiation of rhabdomyosarcomas. Cancer Res. 41: 383-388, 1981.
16. Cottrell, J. C.; Becker, K. L., and Moore, C. F.: Immunofluorescent studies in gonadotropin-secreting bronchogenic carcinoma. Amer. J. Clin. Path. 50: 492-497, 1968.
17. Dutcher, T. F., and Fahey, J. L.: Immunocytochemical demonstration of intranuclear localization of 552 gamma macroglobulin in macroglobulinemia of Waldenström. Proc. Soc. Exp. Biol. Med. 105: 450-455, 1960.
18. Rosai, J., and Rodrigues, H. A.: Application of electron microscopy to the differential diagnosis of tumors. Amer. J. Clin. Path. 50: 555-560, 1968.
19. Murray, M. R., and Stout, A. P.: Tissue culture in tumor classification and diagnosis. In: Pack, G. T., and Ariel, J. M. (eds.), Treatment of Cancer and Allied Diseases. Vol. 1. New York: Hoeber-Harper, 1968. Pp. 124-139.