Histochemistry in the Diagnosis of Malignant Mesothelioma *

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ABSTRACT

To improve the accuracy of diagnosis, 82 cases of malignant mesothelioma were studied for mucosubstance production by the periodic acid-Schiff reaction after diastase digestion (DPAS) and the colloidal iron (CI) reaction before and after hyaluronidase. Twenty-six carcinomas of various origins, similarly treated, were compared. The tumors had been fixed and processed in routine fashion. The reactions were regarded as positive only when there was a significant number of stained cytoplasmic vacuoles and/or stained secretion in tubular or glandular lumina or in spaces between epithelial cells. Scattered intracytoplasmic granules and positive material in the stroma or in mesenchymal tumors were not considered diagnostic.

Approximately half of the mesotheliomas were CI positive. All but two showed complete or partial removal of the CI positive secretion by hyaluronidase. Thus, almost half of the mesotheliomas were demonstrated to contain hyaluronic acid and almost 33 percent of these were shown to contain another type of acid mucosubstance as well. A parallelism appeared to exist between the degree of histologic differentiation and the production of hyaluronic acid. The production of neutral mucosubstance, as indicated by the DPAS, was not conclusively demonstrated in any mesothelioma. Each staining method showed a small number of equivocal reactions. Twelve of 26 carcinomas were DPAS and CI positive but hyaluronidase had no effect on the CI reaction.

The necessity for the accurate diagnosis of malignant mesothelioma has assumed an added dimension because of its statistically significant association with exposure to asbestos, and its apparent increased incidence in recent years. In addition to the usual desirability of precise tumor diagnosis for prognostic and therapeutic purposes, there are epidemiologic and legal considerations with mesothelioma.

The difficulty of diagnosis of mesothelioma is compounded, above and beyond the

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usual problem of anaplasia, by its many variations on an underlying theme of dimorphism. Although familiarity with the more frequently occurring histologic patterns increases the accuracy of diagnosis, a strong element of observer subjectivity remains. Therefore any method for developing more objective data is desirable.

The production of the acid mucosubstance, hyaluronic acid, by many mesotheliomas has been studied by a number of investigators for possible diagnostic applicability. Their reports have not been conclusive as to actual utility with routinely fixed and processed tissue. The opportunity for special fixation and processing does not frequently present itself.

Methods for demonstration of the absence of neutral mucosubstance, to assist in the exclusion of carcinoma, have been more widely applied in practice.

In the course of studying a relatively large series of mesotheliomas, it has been attempted to evaluate the diagnostic usefulness of mucosubstance demonstration and this presentation is based on our experience.

Methods and Materials

This study was carried out on the level of routine diagnostic practicality. The material was fixed in formalin, with the exception of a few cases from Bouin's solution, and was embedded in paraffin in the usual manner. Staining techniques were limited to the periodic acid-Schiff reaction (PAS), according to McManus, after exposure of the sections to diastase (DPAS) and the Hale colloidal iron stain as modified by Rinehart and Abul-Haj before (CI) and after the application of testicular hyaluronidase (HCl). The difference between the CI and HCl preparations was taken as an indication of the extent to which any acid mucosubstance present consisted of hyaluronic acid.

From a group of over 200 cases diagnosed as mesothelioma, pleural and peritoneal, material was available from 82 patients for the application of the methods mentioned. All the cases used were of the epithelial, mixed or biphasic types. Since hyaluronic acid production by pure mesenchymal or sarcomatoid mesotheliomas does not assist in differentiation from sarcomas of other origins, no tumors of this type were included. Approximately 10 percent of the tumors in our total series were purely mesenchymal.

In conformity with the principle of grading mesotheliomas by degrees of certainty of diagnosis, the cases were divided into three categories: (1) definite, (2) probable and (3) possible. The grading was based on the extent to which the tumor corresponded to well-defined histologic patterns as described by numerous authors including Churg, Rosen and Moolten, and McCaughy. Those in the definite group had characteristic patterns while those in the probable group had some of the characteristic features although others were discrepant or lacking. The tumors in the possible group included atypical or nonspecific appearances which could not be assigned to another entity. The evaluation was based on hematoxylin and eosin sections without reference to the histochemical methods. The cases used were taken from each group in random fashion.

For purposes of comparison, 26 carcinomas were similarly studied. Fourteen were adenocarcinomas, seven arising in the lung and one possibly coming from the lung. The others were solid carcinomas of which four were of pulmonary origin and one was possibly of such origin. The comparison of pulmonary carcinomas was believed relevant since a peripheral carcinoma spreading on the pleura is one of the more frequent imitators of mesothelioma. The other adenocarcinomas were from the gastrointestinal tract, breast and ovary.
TABLE I
Histochemical Reactions of 82 Mesotheliomas and 26 Carcinomas

<table>
<thead>
<tr>
<th></th>
<th>Mesotheliomas</th>
<th>Carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Definite 30</td>
<td>Probable 24</td>
</tr>
<tr>
<td>DPAS Positive</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DPAS Questionable</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>CI Positive</td>
<td>23 (77%)</td>
<td>13 (54%)</td>
</tr>
<tr>
<td>CI Questionable</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Results

Presented in table I are data showing that 74 of 82 mesotheliomas showed no reactivity to DPAS. Eight cases presented a minimal or questionable reactivity. This was not the fine granularity described by Fisher and Hellstrom. The latter was present to a varying degree in a number of cases but could not be related diagnostically. No instance of unequivocal DPAS positivity was found. The DPAS was regarded as questionable when there was a distinct granular fuchsinophilic coloration of an occasional cell, and/or a rare vacuole rimmed by positively staining material or a small globule. In several cases, thinner than average sections, meticulously stained, dispelled the illusion of DPAS positivity. Brightly stained stroma insinuated between tumor cells occasionally simulated droplets of secretion.

The importance of preliminary diastase digestion needs no emphasis. Many mesotheliomas have a copious glycogen content. A case was accepted as CI positive only where there was at least a moderate number of stained vacuoles. The rimming of cell borders, diffuse cytoplasmic staining or the presence of cytoplasmic granules were considered non-specific and an infrequent positive vacuole or globule as inconclusive. In some cases, the acid mucosubstance was present in coalescent vacuoles, in the lumen of the tubules seen in many mesotheliomas, in clefts between epithelial cells and in pools in those mesotheliomas with scattered epithelial cells and a myxoid intercellular substance. Staining of the connective tissue stroma was not regarded as diagnostically significant.

If the CI preparation was judged positive by the above criteria, it was compared with the HCI preparation and the extent of disappearance of the CI stained material determined.

In table I are disclosed data that 52 percent of the entire series of 82 cases were CI positive. The frequency of positivity ranged from 77 percent in the definite group through 54 percent of the probable to 21 percent of the possible. Although no attempt was made to quantitate the CI positivity in precise fashion, it was noted that all the strongly reacting cases, i.e., showing the largest amounts of stainable materials, were in the definite group.

Six cases were inconclusive by the CI method. Four of these were in the possible category, as compared with only 6 positives in this group.

In table II, it can be seen that 40 of 42 positive cases showed a total or partial disappearance of CI positive material after hyaluronidase treatment. In 27 (64 percent) disappearance was complete. In two cases no change was detectable following hyaluronidase. These, added to 13 cases that showed incomplete removal, make a total of about 33 percent of the CI positive cases that appear to contain an acid mucosubstance other than hyaluronic acid.

The carcinomas that were CI positive were DPAS positive with one exception. CI
staining was in most instances more extensive than the DPAS. Hyaluronidase had no effect on these.

Discussion

Our experience indicates that histochemical methods, under routine conditions, are not the solution to all the problems of the diagnosis of mesothelioma. However, it would appear that they can be useful in many instances.

In the definite cases where it is not necessary for diagnosis but where it is most likely to be positive, and most strongly positive, the demonstration of hyaluronic acid serves as confirmation. In those cases evaluated as merely possible, where the demonstration of hyaluronic acid would be most helpful, about 20 percent of cases are positive. In this category of uncertainty, a positive DPAS (always accompanied, in our experience, by acid mucosubstance of hyaluronidase resistant character) was helpful in ruling out mesothelioma in favor of a carcinoma. It may be mentioned that some of the mesotheliomas that showed a questionable DPAS also showed acid mucosubstances that were only in part hyaluronic acid. The CI staining in these cases, especially when less marked, must be evaluated with caution.

In the intermediate (probable) group, half the cases contained hyaluronic acid and here the information is certainly of help in diagnosis.

The absence of demonstrable acid mucosubstance in almost half the total series may be attributed either to lack of its synthesis or dissolution in fixation and/or processing.

In this study and discussion, terms mucosubstance and hyaluronic acid have been employed in a purely histochemical sense. Spicer, Horn and Leppi have referred to the lack of identity of the histochemical and biochemical characterization of mucopolysaccharides. What has been referred to as hyaluronic acid is the colloidal iron positive material sensitive to testicular hyaluronidase. Testicular hyaluronidase is known to be able also to remove certain chondroitin sulfates. However, the definite characterization of the acid mucopolysaccharides of mesotheliomas was not the intent of this study.

The identity of the DPAS positive material seen in small amounts in some mesotheliomas also was not established. It was not of such quantity or character in any of the cases as to resemble that seen in adenocarcinomas. This matter is discussed in the classic paper of Wagner, Munday and Harington.

A final point of more general interest is the parallelism of the degrees of diagnostic certainty and the presence of hyaluronic acid. On a practical level, this would seem to substantiate the validity of the histologic criteria for the diagnosis. On a more theoretic level, this would seem to suggest a correlation between differentiation and the ability, qualitatively and quantitatively, to produce hyaluronic acid.

### TABLE II

**Reaction with Colloidal Iron of Mesotheliomas and Carcinomas after Hyaluronidase**

<table>
<thead>
<tr>
<th>CI Positive</th>
<th>Definite</th>
<th>Probable</th>
<th>Possible</th>
<th>Carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMP Totally Removed</td>
<td>17</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>HCl AMP Diminished</td>
<td>5</td>
<td>7</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>AMP Unchanged</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>13</td>
</tr>
</tbody>
</table>
References


