HYALURONIC ACID IN THE PLEURAL FLUID ASSOCIATED WITH A MALIGNANT TUMOR INVOLVING THE PLEURA AND PERITONEUM*

BY KARL MEYER AND ELEANOR CHAFFEE

(From the Department of Ophthalmology, College of Physicians and Surgeons, Columbia University, and the Institute of Ophthalmology, Presbyterian Hospital, New York)

(Received for publication, December 22, 1939)

Mucilaginous tumors and tumor fluids occur quite frequently. The nature of the mucilaginous constituent, however, has been determined in only one instance: Kabat (2) isolated a viscous polysaccharide acid from the tissues and a cyst of a tumor of a filtrable fowl sarcoma. This polysaccharide was shown by its chemical properties and by enzymatic hydrolysis to be very similar to, if not identical with, hyaluronic acid which has been found in cattle and pig vitreous humor (3, 4), human umbilical cord (3), cattle and human synovial fluid (5), and Group A hemolytic streptococci in the mucoid phase (6). Probably the mucoid capsule of Group C hemolytic streptococci also contains hyaluronic acid (7).

In this paper the isolation of hyaluronic acid from the viscous pleural fluid of a patient with a malignant tumor of the pleura and peritoneum is reported. The fluid was obtained through the courtesy of Dr. R. Loeb of the Department of Medicine. Clinically and pathologically the tumor was diagnosed as a mesothelioma or endothelioma. The peritoneal fluid had the same appearance as the chest fluid. It is quite obvious that the tumor cells themselves are the source of the hyaluronic acid, for this type of tumor frequently produces viscous fluids in the serous cavities (8). Furthermore, the usual transudates and exudates of the pleura and peritoneum are not viscous, even when a malignant tumor exists in these regions. Another indication is the almost constant

* A preliminary report of this work has been published (1).
Hyaluronic Acid in Pleural Fluid

yield of hyaluronic acid in the fluids taken at different time intervals.

EXPERIMENTAL

Three different samples of pleural fluid were obtained. The procedure used for the isolation of the polysaccharide acid was similar to that used for the isolation of hyaluronic acid from synovial fluid (5), except that a final precipitation of the material in 10 per cent acetic acid by 2 volumes of alcohol was substituted for the glacial acetic acid precipitation previously used. There-

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Nitrogen</th>
<th>Hexosamine</th>
<th>Uronic acid</th>
<th>Acetyl</th>
<th>Acid equivalent weight</th>
<th>$[\alpha]_D$</th>
<th>Moisture</th>
<th>Ash</th>
<th>Equivalents per equivalent weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.21</td>
<td>39.6</td>
<td>44.9</td>
<td>9.85</td>
<td>622</td>
<td>-76.8</td>
<td>1.27</td>
<td>4.04</td>
<td>1.43</td>
</tr>
<tr>
<td>2</td>
<td>3.00</td>
<td>40.8</td>
<td>46.6</td>
<td>10.5</td>
<td>576</td>
<td>-70.3</td>
<td>6.67</td>
<td>2.16</td>
<td>1.23</td>
</tr>
<tr>
<td>3</td>
<td>2.95</td>
<td>40.0</td>
<td>46.7</td>
<td>10.3</td>
<td>641</td>
<td>-67.7</td>
<td>3.15</td>
<td>1.44</td>
<td>1.35</td>
</tr>
<tr>
<td>Synovial*</td>
<td>3.42</td>
<td>39.1</td>
<td>42.5</td>
<td>10.2</td>
<td>477</td>
<td>-73.3</td>
<td>1.17</td>
<td>1.04</td>
<td>1.04</td>
</tr>
</tbody>
</table>

* Average of six samples.

Therefore a mixture of the free acid and its calcium salt was obtained, as the analysis of the material and its ash showed. Table I gives the analyses of the three samples isolated compared with the average data of six samples of hyaluronic acid prepared from cattle synovial fluid.

The acid equivalent weights are too high and therefore the equivalents per equivalent weight are too high. But Table I shows that, in the limits of the error of the methods, the acid is composed of equimolar parts of hexosamine, hexuronic acid, and acetyl, and the rotation is very similar to that of the synovial fluid polysaccharide.
The hexosamine was isolated as the hydrochloride, as described earlier (3). 110 mg. were obtained from 300 mg. of polysaccharide or 82 per cent of the amount determined analytically. The analysis was as follows: N (Dumas) 6.43 per cent, Cl (by titration) 16.1 per cent (theoretical N 6.49 per cent, Cl 16.4 per cent), initial \([\alpha]_D +103^\circ\), at equilibrium \(+71.4^\circ\). The compound was therefore glucosamine hydrochloride.

The identity of the pleural fluid polysaccharide with hyaluronic acid was finally established by enzymatic analysis. The enzyme was prepared from a rough pneumococcus designated as Strain D-39-R. Its preparation and properties are described elsewhere (9). The enzyme is highly specific for hyaluronic acid. The kinetics of hydrolysis of a sulfonated derivative of hyaluronic acid are, for example, quite different from that of the original sample.¹

For comparison five different enzyme concentrations were incubated at 37° at pH 5.9, in the presence of toluene, with 5 mg. of the polysaccharides from the tumor fluid and from umbilical cord. Reducing values determined at 2 and 20 hours are plotted in Fig. 1. It is evident that the curves agree closely and from this agreement it is concluded that the polysaccharides are identical.

The yield of polysaccharide per 100 cc. of pleural fluid corresponded to 0.174, 0.187, and 0.142 per cent as compared to 0.02 to 0.025 per cent from bovine synovial fluid, about 0.04 per cent from bovine vitreous humor, and 0.130 per cent from the cyst fluid investigated by Kabat.

In previous papers from this laboratory it was pointed out that hyaluronic acid occurred free or in salt linkage only in vitreous humor and synovial fluid and was not chemically bound to protein (3, 10, 11). On the other hand, protein complexes are formed by the polysaccharide acids on acidification in the presence of protein. Such protein complexes were shown to be salts formed by the combination between the acid group of the polysaccharide and the basic groups of the proteins in stoichiometric proportions. The so called "mucins" prepared by acidification of the native fluid must therefore be considered artifacts.

¹ Only one polysaccharide acid not containing glucuronic acid has been found which is also split by the enzyme though at a markedly different rate. The latter was isolated from submaxillary gland (unpublished).
To test for the presence of protein compounds, samples of (a) the pleural fluid (Sample 1), (b) the polysaccharide isolated from it, (c) the polysaccharide from umbilical cord, and (d) an acid polysaccharide in a firm chemical linkage with a polypeptide were submitted to Dr. Longsworth of the Rockefeller Institute for electrophoretic analysis.

The pleural fluid was centrifuged to clear it of cells, diluted with an equal volume of veronal buffer of pH 7.8, dialyzed against the buffer, and analyzed as described by Longsworth and co-workers (12). Four components could be distinguished. The fastest component had a mobility of $-10.5 \times 10^{-6}$, and the second and main component a mobility of $-5.6 \times 10^{-5}$, which is similar to that of albumin in serum. Moreover, the yellow pigment of

---

\(2\) We are greatly indebted to Dr. Longsworth for carrying out these experiments.
the exudate migrated with this second component. This is similar to the migration of bilirubin with serum albumin and suggests that the second component of the chest fluid is serum albumin. The third and fourth components have mobilities of −2.9 and −0.5 respectively, similar to the β- and γ-globulins of serum. No peak which might correspond to α-globulin was evident in the pattern. This is probably due to the fact that, owing to the high mobility of the first component, the electrolysis was interrupted too early for this component to separate from the albumin. The total area of the pattern corresponded to the following values, first component 5 per cent, second component 65 per cent, third component 13 per cent, fourth component 17 per cent.

From the nitrogen value of the fluid, minus non-protein nitrogen, minus glucosamine nitrogen of the isolated polysaccharide, the total protein in the fluid was calculated as 3.55 per cent. From this figure and from the relative concentration of the first component, a concentration in the original fluid of this component was calculated as 0.17 per cent, while the amount isolated by us corresponded to 0.174 per cent. The close agreement between these figures indicates that there was migration of the free polysaccharide rather than of a protein complex. This is borne out by the mobility of the isolated polysaccharide acids at the same pH. The polysaccharides of the tumor and the umbilical cord both had a mobility of $-10.7 \times 10^{-5}$, while the peptide complex of an acid polysaccharide (which, however, contains a hexonic acid instead of glucuronic acid) had a mobility of $-6.95 \times 10^{-5}$. The close agreement between the mobility of the fast component in the tumor fluid and of the isolated polysaccharides seems to prove that the fast component in the fluid is the free polysaccharide acid.

Hyaluronic acid or its salts in solution always show a marked viscosity. However, the data on solutions from different sources and by different investigators have varied considerably. As a rule the viscosity varied inversely with the purity of the preparations. In the case of synovial fluid, though no component of marked viscosity could be found other than hyaluronic acid, nevertheless the isolated polysaccharide accounted for only a fraction of the viscosity of the fluid from which it was prepared.

A very great discrepancy was found in the present case. The relative viscosity of the native fluid centrifuged at 4000 r.p.m.
was 147.6 at 20° (relative to 0.9 per cent sodium chloride), while the neutralized polysaccharide at a corresponding concentration of 0.177 per cent in 0.9 per cent sodium chloride had a viscosity of only 1.54. (The viscosity of a 0.25 per cent solution, the concentration used in work previously published, was 1.79 (3, 5).) In Fig. 2 the relative viscosities of the pleural fluid have been plotted against the dilutions of the fluid with 0.9 per cent sodium chloride.

![Graph](https://via.placeholder.com/150)

**Fig. 2.** Relative viscosity of pleural fluid at different dilutions

The great drop in viscosity obviously cannot be explained by the assumption of a true solution of fibrous macromolecules.

**DISCUSSION**

From the drop of the viscosity on dilution and the normal conductance of the dialyzed and diluted fluid (reported by Dr. Longsworth), it must be concluded that the original fluid is a gel formed by the polysaccharide acid and the copresent protein. This con-
clusion is further borne out by the fact that the fluid shows thixotropy and that air bubbles in the fluid assume an ellipsoidal shape, both factors typical for a gel (13). The great variations in viscosity of the isolated polysaccharides may also best be explained by gel formation. The gel formation of the polysaccharide acid in the capsule of the hemolytic streptococci is obviously of great biological importance, as is in all likelihood the status of the polysaccharide acid in the vitreous humor.

A mobility similar to the one in the present case has been observed by Hesselvik in filtered vitreous humor (14). The fastest component had a mobility of \(-11.4 \times 10^{-5}\), which the author ascribed to "hyalomucoid." The difference between this and the mobility of \(-10.5 \times 10^{-5}\) measured by Longsworth is probably due to difference in buffers and in ionic strength. Obviously the Upsala investigator was likewise measuring the mobility of hyaluronic acid.

Hyaluronic acid in the capsule of hemolytic streptococci is connected with the virulence of the organisms. The virulent forms have been shown to possess mucoid capsules (15). Moreover the appearance in the organisms of an enzyme hydrolyzing hyaluronic acid is apparently somehow connected with the loss of virulence (9).3 In Group C hemolytic streptococci good experimental evidence has been brought forward to link the amount of an immunologically inactive acid polysaccharide, which is apparently hyaluronic acid, with the degree of encapsulation, virulence, and invasiveness (7). Another example of the connection between bacterial virulence and a mucilaginous constituent is that of gastric "mucin" which renders virulent for mice (or increases the virulence of) a number of microorganisms avirulent (or of low virulence) for this species (16, 17). This effect of a mucoid capsule is obviously not due to any change inherent in the microorganism, but is probably due to a failure of the natural defense mechanism of the host to cope with the invading organism so that the latter can grow unchecked. It is tempting to speculate in the case of the malignant tumors as to the operation of a similar mechanism in which hyaluronic acid or similar constituents may prevent the action of the normal restraining influences of the host tissues, so

3 Unpublished data.
that the inherent growth tendencies of the tumor tissues become dominant.

SUMMARY

The viscous component of a pleural fluid associated with a malignant tumor of the pleura and peritoneum has been isolated. The substance has been shown to be hyaluronic acid and to be similar in composition and rotation to this polysaccharide obtained from other sources. This was further substantiated by the results of the enzymatic hydrolysis of a pneumococcus enzyme.

In the electrophoresis apparatus a very fast component with a mobility of $-10.5 \times 10^{-5}$ was observed and shown to be free hyaluronic acid. The concentration of this fraction as determined by the electrophoresis experiment was very similar to the concentration of the acid obtained by isolation. Furthermore, the mobility of hyaluronic acid isolated from the tumor fluid and from umbilical cord was $-10.7 \times 10^{-5}$, very similar to that of the fast fraction in the pleural fluid.

The viscosity of the tumor fluid, as measured in an Ostwald viscosimeter, was about 95 times greater than the viscosity of the isolated polysaccharide acid in an equivalent concentration, dropping sharply on dilution. By the abnormal viscosity the fluid is characterized as a gel. Additional evidence for this hypothesis is given.

BIBLIOGRAPHY

HYALURONIC ACID IN THE PLEURAL FLUID ASSOCIATED WITH A MALIGNANT TUMOR INVOLVING THE PLEURA AND PERITONEUM
Karl Meyer and Eleanor Chaffee


Access the most updated version of this article at [http://www.jbc.org/content/133/1/83.citation](http://www.jbc.org/content/133/1/83.citation)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/133/1/83.citation.full.html#ref-list-1](http://www.jbc.org/content/133/1/83.citation.full.html#ref-list-1)