THE DETECTION OF HYALURONIC ACID IN PLEURAL FLUIDS OF CASES WITH DIFFUSE PLEURAL MESOTHELIOMAS

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HYALURONIC acid has been found to be associated with a number of different malignant conditions. Meyer and Chaffee (1940) reported it present, at a concentration of 0·2 per cent, in the pleural fluid of a patient with pleural mesothelioma. Blix (1951) found it at a concentration of 0·7 per cent in pleural fluid taken from a patient with the same condition. In both cases the authors considered that the tumour cells appeared to have produced this tumour fluid. In 1959 Meyer (personal communication) isolated hyaluronic acid from 10 mesothelioma pleural fluids, and more recently, Wagner, Munday and Harington (1962) demonstrated, histochemically, the presence of this polysaccharide in diffuse pleural mesotheliomas, and used its presence to distinguish this condition from peripheral pulmonary adeno-carcinoma. Ropes, Robertson, Rossmeisl, Peabody and Bauer (1947) reported hyaluronic acid, or a polysaccharide very similar to it, in human and rabbit myxoedematous tissues, in the inflamed pleurae of a patient with rheumatoid arthritis and in several transplanted tumours. In 1954 Harris, Malmgren and Sylvén found that Rous fowl sarcoma tissue is rich in hyaluronate. Since then it has been found again in the Rous sarcoma, in human myxoma (Orr, 1954), and in tumours produced by a virus causing fowl sarcoma and leucosis (Kabat, 1939). Pirie also isolated this material, in 1942, from Rous and Fujinami tumours in fowls. Kabat considered it very probable that the tumour cells themselves, rather than the surrounding muscle tissues, produced the polysaccharide.

The present investigation was carried out to supplement the histochemical study of Wagner, Munday and Harington (1962) on mesotheliomatous tissue.

MATERIALS AND METHODS

A total of 207 pleural fluids, ranging in volume from 5 to 1800 ml. were tested for the presence of hyaluronic acid by a chemical method depending upon the isolation of the polysaccharide from the pleural fluid. Although the method may require considerable quantities of pleural fluid, as little as 5 ml. may be suitable if high concentrations of hyaluronic acid are present. Such secretions are extremely viscous when aspirated from the pleural cavity.

A mucin clot test was first employed by diluting the fluids with 2·5 volumes of water and then adding 50 per cent acetic acid : water (vol./vol.) to make a final concentration of 0·2 per cent. This test is only reliable for highly-polymerized hyaluronic acid, and was considered positive if a typical syneretic clot is obtained, and which could be prevented by prior incubation of the fluid with hyaluronidase for 30 min. at 38°.

The stringy clots of crude hyaluronic acid (which floated to the surface of the fluid in the measuring cylinders) were removed with glass rods and dried *in vacuo* over calcium chloride.

They were then digested with pepsin at pH 1 for 24 hr., neutralized to pH 7·4 and incubated with trypsin. This was followed by alcoholic extraction of the polysaccharide from the digest. After drying over calcium chloride the amount of hyaluronic acid present was expressed as a percentage of the pleural fluid used for the test.

In order to confirm that this material consisted predominantly of hyaluronic acid, all samples for each case were pooled and analyzed chemically for nitrogen, hexosamine (Elson and Morgan's method as modified by Boas, 1953), hexuronic acid (Dische, 1947) and for acetyl groups (Ludowieg and Dorfman, 1960). The results thus obtained were similar to those given by standard hyaluronic acid (Nutritional Biochemicals Corporation).

In addition, some of the protein-free clots were dissociated with 5 per cent sodium acetate at pH 8·0, dialyzed, and the solutions submitted to paper electrophoresis after they had been reduced in volume. Bands were observed agreeing with those given by standard hyaluronic acid.

RESULTS

The amounts of hyaluronic acid found in 18 pleural fluids, taken from 6 cases of diffuse pleural mesothelioma are shown in the Table.

THE TABLE—*Percentage of Hyaluronic Acid in the Pleural Fluids of Six Cases of Histochemically-proved Diffuse Pleural Mesothelioma*

Number of specimens	A*	B	C	D	E*	F*
1	0·16	0·15	0·86	0·31	0·012	0·06
2	Nil	0·09	—	0·37	0·098	—
3	0·27	0·12	—	—	—	—
4	0·66	0·3	—	—	—	—
5	0·73	—	—	—	—	—
6	0·79	—	—	—	—	—
7	0·53	—	—	—	—	
8	Nil	—	—	—	—	—

* These patients died shortly after the last specimen was received.

DISCUSSION

The samples of fluids from cases A, B and E were submitted at intervals ranging from 1 to 6 weeks, and in Case D there was an interval of 13 weeks. The table shows that in Cases A, B, D and E the concentration of hyaluronic acid in the pleural fluid generally increased with the progress of the disease. In Case A, where several determinations were made, the concentration reached the very high figure of 0·79 per cent after which it dropped through 0·53 per cent to 0 per cent. This last sudden drop suggests gel formation by the polysaccharide ; hence its possible failure to pass out with the aspirated fluid from the pleural space. The gel would remain there, probably as a mucilaginous clot.

Pleural fluids were examined from cases with idiopathic effusions, tuberculous infections and from malignancies other than mesothelioma of the pleura, but hyaluronic acid was detected only in fluid from cases with the last-named condition. Although no false positives were obtained in the human cases, one was found in a mouse which was later shown to have had a streptococcal infection which accounted for the production of hyaluronic acid. In a rat and a guinea-pig in which experimental pleural mesotheliomas had been confirmed histochemically, hyaluronic acid was found in the pleural fluids at concentrations of 0·02 per cent and 0·019 per cent respectively.

Six cases of histochemically-proven mesotheliomas gave positive results for hyaluronic acid determination, in one other the clots were too small to work with (trace amount and 0·004 per cent), and 27 were negative. All 34 cases had been exposed to crocidotite asbestos. At present the detection of hyaluronic acid in the pleural fluid suggests the presence of a diffuse mesothelioma which should be confirmed by pleural biopsy.

SUMMARY

Hyaluronic acid was isolated in concentrations ranging from 0·012–0·79 per cent from the pleural fluids of 6 histochemically-proven cases of diffuse pleural mesothelioma.

In four cases, the concentration increased with the progress of the disease, and in one of these it dropped rapidly before death. It was presumed that this was due to gel formation by the polysaccharide.

The detection of hyaluronic acid in the pleural fluid is a useful aid to the diagnosis of diffuse pleural mesotheliomas.

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REFERENCES

Blix, G.—(1951) *Acta Soc. Med. Upsalien*, **56**, 47.
Boas, N. F.—(1953) *J. biol. Chem.*, **204**, 553.
Dische, Z.—(1947) *Ibid.*, **167**, 189.
Harris, R. J. C., Malmgren, H. and Sylvén, B.—(1954) *Brit. J. Cancer*, **8**, 141.
Kabat, E. A.—(1939) *J. biol. Chem.*, **130**, 143.
Ludowieg, J. and Dorfman, A.—(1960) *Biochim. biophys. Acta*, **38**, 212.
Meyer, K. and Chaffee, E.—(1940) *J. biol. Chem.*, **133**, 83.
Orr, S. F. D.—(1954) *Biochim. biophys. Acta*, **14**, 173.
Pirie, A.—(1942) *Brit. J. exp. Path.*, **23**, 277.
Ropes, M. W., Robertson, W. v. B., Rossmeisl, E. C., Peabody, R. B. and Bauer, W.—(1947) *Acta med. scand.*, supp. 196, 700.
Wagner, J. C., Munday, D. and Harington, J. S.—(1962) *J. Path. Bact.*, **84**, 73.