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BIOCHEMICAL STUDY ON MUCINOUS PLEURAL EFFUSION IN A CASE OF ALVEOLAR CELL CARCINOMA OF THE LUNG

Part I. Analytical data on the effusion in a raw state

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ABSTRACT—— In a case of alveolar cell carcinoma of the lung with mucinous pleural effusion, a biochemical study was conducted to elucidate the chemical nature of the mucin.

Carbohydrate analysis of the effusion revealed an increased content of protein-bound hexose, especially in a form of so-called mucoprotein hexose. Ouchterlony's method disclosed the presence of serum protein components in the fluid. Electrophoretic study could not separate mucinous principle because of the high viscosity of the fluid. Hyaluronidase eliminated high viscosity of the fluid, and also high content of hyaluronic acid in the fluid was observed.

Some tumors have activity of producing mucin. But this substance has been rather rarely a subject for chemical analysis. A few chemical studies[1-5] carried out from the viewpoint of glycoprotein or mucopolysaccharide have been published on bronchial secreta or pleural effusion in patients with lung cancer. Gernez-Rieux and his associates analysed sputa from patients with alveolar cell carcinoma of the lung, a tumor with remarkable activity of producing mucin, and they concluded that the sputa contained sialic acid in high ratio and serum protein components.

The authors recently experienced an unusual case of alveolar cell carcinoma of the lung in which pleural effusion was characterized by extremely high viscosity and by formation of mucin clot in dilute acetic acid. Clinical and pathological findings will be published elsewhere.[6] Attempts have been made to separate the substance causing high viscosity and elucidate its chemical properties. The present paper deals with the results of chemical analysis on raw effusion obtained from the patient.

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In some of pulmonary or pleural tumors, such as mesothelioma, a few authors[7-11] observed highly viscous pleural effusion which contained hyaluronic acid in high concentration. Detailed study on this substance in the present case will be the theme of further investigation.

MATERIALS AND METHODS

Materials: Each sample of pleural fluid obtained intra vitam was immediately frozen and stored in a freezer, divided into aliquots or as its whole, until it was used. Repeated freezing and thawing was avoided because it was found that values of protein-bound hexose decreased through these procedures. Approximately 5 l in total were drawn out in quantity of 300 to 500 ml at each tapping, of which about 3 l were spared and stored. Thick viscous fluid was found in the left pleural cavity on autopsy and was also frozen and stored. Before analysis, thawed material was centrifuged at 4,000 rpm for 30 min at 4 C to remove cellular debris and subjected to use.

Methods:

I) Rough fat content: The thawed material was diluted with an equal volume of physiological saline. The diluted fluid was vigorously shaken in 20 volumes of chloroform-methanol mixture(2:1, v/v) and then let standing still overnight. The solvent layer was collected and filtered through a filter paper (Toyo filter paper No. 51). After several washings with one quarter volume of distilled water the extract was dried and then the residue was weighed.

II) Protein content: Estimated by a refractometer.

III) Nitrogen content: Estimated by a micro-Kjeldahl's method.

IV) Carbohydrate content:

1) Protein-bound hexose: Precipitation procedures were carried out by the method of Weimer and Moshin.[12] Winzler's precipitation procedure[13] was applied for so-called mucoprotein hexose. Orcinol-sulfuric acid reaction was used for colorimetric determination according to Winzler.[13]

2) Methylpentose: Dische and Shettles' cystein-sulfuric acid reaction[14] was used, adopting 10 min for boiling time.

3) Hexosamine: Elson-Morgan reaction was applied according to Winzler.[13]

4) Sialic acid: For rough estimation, DPA reaction[13] was used without any correction. And for more precise value, resorcinol reaction of Svennerholm[15] was used.

5) Hexuronic acid: Quantitative study was not carried out. For qualitative purpose, Fishman reaction was used.

6) Hyaluronic acid: Estimated according to the method of Jensen.[17]

V) Enzymatic degradation by hyaluronidase: Hyaluronidase was obtained in commercially available pharmaceutical product by Takeda Pharmaceutical Company (Halodase). Incubation was carried out at 37 C for 30 min.

VI) Electrophoresis:

1) Paper electrophoresis: It was done on Toyo filter paper No. 51 in veronal buffer

of pH 8.6 (μ=0.05).

2) Cellulose acetate electrophoresis: Cellulose acetate membrane was used in buffer with the constitution above described.

3) Free electrophoresis: Using a Tiselius apparatus (Hitachi HTD 1), it was performed at various pH's of buffer solution.

VII) Immunodiffusion: Ouchterlony's standard procedure was carried out employing commercially available antibody solution (Behringwerke).

RESULTS

1) Physical characteristics: The effusion was brownish yellow, semitransparent or slightly turbid fluid with extremely high viscosity. In the earlier date of the clinical course, tapped fluid did not flow out from test tube even when it was upside down. It did not coagulate though fibrin net was formed. Anticoagulant such as potassium oxalate had no influence on the viscosity. Pleural fluid obtained at autopsy was more mucinous and contaminated by destroyed tissue but basically similar to the punctate. The punctate was not completely homogenous, as it was noted that white less transparent stringy substance was rather unevenly dispersed, especially in material obtained at autopsy. But most of the fluid was also viscous and stringy.

Specific gravity of the fluid could not be determined by a hydrometer because of its high viscosity. By pycnometer, it was 1.019 at 13 C.

II) Chemical data: When dropped into dilute acetic acid solution, the punctate formed thread-like white amorphous mass, i. e. mucin clot, which was dissolved out when alkaline solution was further added.

Analytical data are shown in Table 1.

Electrophoresis failed to achieve complete separation of mucinous substance. On electrophoresis, viscous substance remained at the original point of run and was interfered with movement of other components. Only free electrophoresis could disclose the

Table 1. Analytical data of raw effusion

	Value
Dry weight(%)	5.5
Protein(%)	2-3
Nitrogen(%)	4.9
Fat(mg/dl)	250
Total protein-bound hexose (mg/dl, Winzler)	140
Mucoprotein hexose (mg/dl, Winzler)	105
Methlpentose (mg/dl)	8-10
Hexosamine (mg/dl)	184
DPA reacting substance (mg/dl)	38.8
Hyaluronic acid (mg/dl)	ca. 400
Hexuronic acid	+
Serum protein components	+

fluid was composed of two constituents.

Immunodiffusion disclosed the presence of serum protein components in the effusion.

When the effusion incubated with hyaluronidase, its viscosity decreased remarkably and finally to that of common effusion. Quantitative study was not carried out.

Hyaluronic acid in the material recovered at autopsy was estimated to be 400 to 500 mg/dl.

DISCUSSION

The pleural effusion in the present case was characterized by extremely high viscosity and by formation of mucin clot in dilute acetic acid. The pleural fluid with these characteristics has rarely appeared in the literature.

As high viscosity in biological material and mucin clot formation are characteristics of high molecular carbohydrate compounds such as glycoprotein or hyaluronic acid,[18] the effusion was analysed to determine carbohydrate contents. Analytical data revealed high content of protein-bound hexose(Table 1). As to values of carbohydrate compounds other than hexose, available data for comparison were very limited in the literature. Comparative study is being continued in our laboratory.

Immunodiffusion study disclosed the presence of serum protein components in the effusion. Some authors[19] found good correlation between mucoprotein value and alpha-2-globulin in effusion. But in the present case, ratio of mucoprotein hexose to total protein-bound hexose value was much higher than would be found in any serum or exudate. Probably, the values of so-called mucoprotein hexose in the pleural fluid presently described included those derived from other substances than orosomucoid. Further detailed analytical studies are necessary to clarify this point.

Exudate with high viscosity and high hyaluronic acid content has been reported on by several authors. From the evidence that incubation of the fluid with hyaluronidase resulted in a significant decrease in viscosity, and that high content of hyaluronic acid was found in the present case, it was assumed that the mucinous principle might be hyaluronic acid. Detailed study on this substance will be published soon.

SUMMARY

Analytical data on mucinous pleural effusion in a raw state were presented. Carbohydrate analysis revealed increased content of protein-bound hexose, especially in a form of so-called mucoprotein hexose. Immunodiffusion revealed the presence of serum protein components. Electrophoretical study could not separate mucinous principle. Addition of hyaluronidase to the fluid eliminated its high viscosity, and hyaluronate content was high.

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