

Clinical and Experimental Studies on the Significance of the Forms of Polarographic Protein Double Wave. (III)

On the Electrophoretically Separated Mucoprotein

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Received September 19, 1959

In the previous paper¹⁾²⁾, the authors reported that the first maximum of protein double wave taken from serum mucoprotein was closely related to protein-bound polysaccharides from chemical and clinical point of view. It was also pointed out that in patients with acute leukemia the first maximum of Brdicka's filtrate wave was always higher than the second maximum.

As was already reported by Peterman, Hogness,³⁾ Mehl, Winzler,⁴⁾ Kalous,⁵⁾ Markham, Jacob, Fetcher⁶⁾ and other investigators, serum mucoprotein could be electrophoretically separated into two components (M_1 , M_2), when serum was run in acid buffer of pH 4.4-4.6.

In this paper further efforts were made to find out the polarographic characteristics of electrophoretically separated mucoprotein with special reference to the wave form.

MATERIAL AND METHOD

Studies were carried out on subjects with leukemia, cancer and other diseases and on healthy controls.

Electrophoresis : 0.05ml of serum was applied to the anodic end of the seven Toyoroshi No. 51 paper strips, 2cm wide, mounted in one cell, and electrophoresis was carried out against the Mc Ilvaine buffer of pH 4.4 and ionic strength of 0.2 for 16 hours or against the veronal buffer of pH 8.6 and ionic strength 0.075 for 10 hours at 200 volts.

Stain : Immediately after electrophoresis two out of seven strips were oven-dried for 30 minutes at 100°C, and one was stained with amido black 10B stain, and the other with PAS stain (Laurell Skoog's⁷⁾ modification method).

Elution : The remaining five strips were not stained but were each cut into 4 or 5 segments corresponding to each protein spot already stained with amido black 10B stain : four segments in acid buffer, namely M_1 , M_2 , A, B and five segments in veronal buffer, namely albumin, α_1 -, α_2 -, β -, γ - globulin. Then the same segments from five different paper strips were respectively collected into one jar. 2.5 ml of physiological saline solution was poured into each of the jars. These were placed in a refrigerator for 12 hours.

Sulfosalicylic acid filtrate : To 1.0 ml of each eluate was added 0.5 ml of 20%

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sulfosalicylic acid, kept for 10 minutes at room temperature and subsequently centrifuged for 15 minutes at 3000 rpm. This supernatant fluid and the eluate were each submitted to polarographic examination under the same conditions, as previously reported, with special caution to keep the constant temperature (25°C) through the procedure.

Polarography : Into each electrolysis cell was poured 1.0 ml of trivalent cobalt test solution which was prepared as follows :

- A) 2-N ammonium chloride solution,
- B) $2 \times 10^{-2} M$ hexamincobalt chloride (luteosalt) solution,
- C) 2-N ammonium hydroxide solution.

The test solution was prepared by adding just before the experiments in the following order : 1 vol. of A, 1 vol. of B, 8 vol. of C. To each cell was added 1.0 ml of each of the 3 or 4 eluates or 1.0 ml of each of sulfosalicylic acid supernatant fluids.

When the wave height was higher than 70 mm, sample was diluted with some volume of cobalt test solution (which was made at half concentration of above described test solution) to lower the wave height to about 70mm. Then dilution rate and wave height were calculated as follows :

dilution rate = $2 + \text{volume (ml) of added test solution}$,

wave height = $70 \text{ mm} \times 1/2 \times \text{dilution rate}$.

Brdicka's test: Ordinary Brdicka's filtrate test with each serum was simultaneously carried out.

RESULTS

(1) Analysis of Brdicka's filtrate test by paper-electrophoresis technique : Each wave height of electrophoretically isolated protein fractions was compared with that of SSA filtrate, namely, M_1 wave with filtrate wave of M_1 , albumin wave with filtrate wave of albumin and so on (Table 1).

Table 1. Comparison between the native value and sulfosalicylic acid filtrate value in respect to electrophoretically separated fractions.

| | | Mc Ilvaine buffer of pH 4.4 | | | | Veronal buffer of pH 8.6 | | | | |
|------------------|----------------|-----------------------------|-------|------|------|--------------------------|------------|------------|---------|----------|
| | | M_1 | M_2 | A | B | Al. | α_1 | α_2 | β | γ |
| Wave height (mm) | A Native | 70 | 170 | 5610 | 1050 | 4900 | 290 | 490 | 560 | 770 |
| | B SSA filtrate | 66 | 105 | 105 | 10 | 70 | 200 | 310 | 50 | 10 |
| | B/A (%) | 95 | 62 | 1.9 | 0.96 | 1.4 | 69 | 63 | 9 | 1.3 |

As shown in this Table, it is evident that all fractions participate more or less in making the Brdicka's filtrate wave in the following, M_1 , M_2 , A and B in acid buffer or albumin, α_1 , α_2 , β , γ globulin in veronal buffer.

In other words, Brdicka's filtrate test originates in protein mixture where

NOTE

M_1 and nextly M_2 or α_1 and nextly α_2 -fractions play chief role.

(2) Analysis of wave from: In Fig 1 is shown the wave forms of each

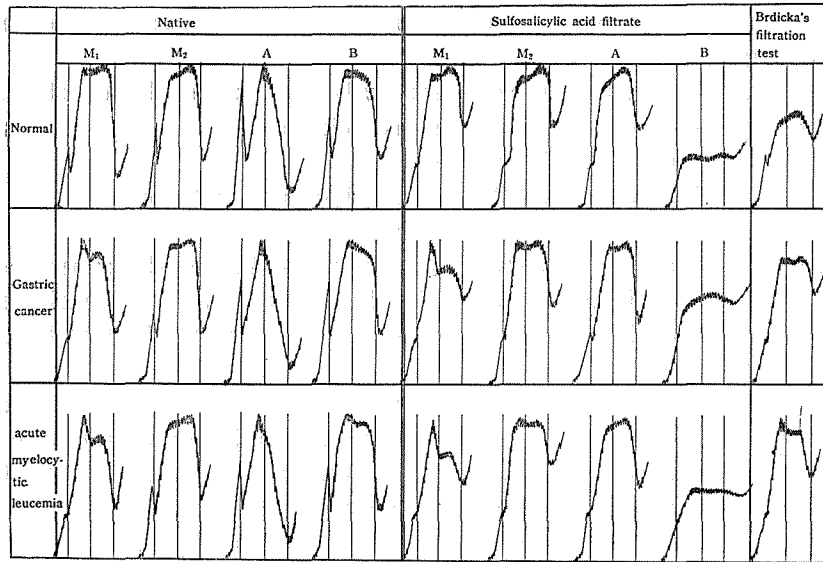


Fig. 1. Paper electrophoresis-polarogram of serum protein.

fraction at the same wave height. Here M_1 fractions, especially those in patients with leukemia or cancer, show the most characteristic wave with markedly prominent first maximum which has never been seen on ordinary Brdicka's filtrate wave.

However, as previously reported (Part II), in acute leukemia the tendency of the elevation of first maximum was also seen in Brdicka's filtrate test. Consequently, it is also demonstrated from the view-point of wave form that Brdicka's filtrate test is caused by the protein mixture in which M_1 fraction takes the greater part.

(3) Relation of PAS stain to amido black 10B stain: The rate of PAS stain to amido black stain was always higher in M_1 than in M_2 fraction. Especially this rate from the M_1 fraction in patient with acute leukemia was the highest.

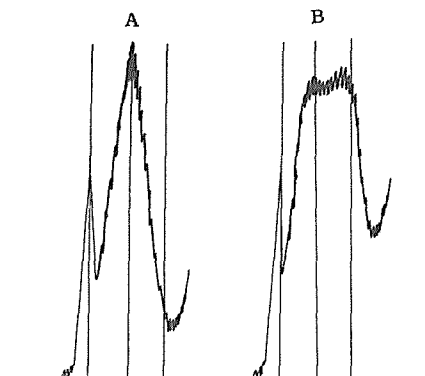
COMMENT

There are many factors which affect the wave form. Among them the primary one is the composition of cobaltic ammonium buffer. When the concentration of NH_3 in buffer is increased the first maximum of double wave, in comparison with the second maximum, elevates considerably.

In fact, the albumin wave, shown in Fig. 1 (A fraction), is seen as having only the first maximum because of the relatively high concentration of NH_3 .

The albumin waves taken in the different NH_3 concentration are shown in Fig. 2.

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A, $10^{-1}M$ NH in buffer. B. $10^{-2}M$ NH in buffer.
Fig. 2. Albumin wave in different buffer.

Table 2. Influence of temperature on the wave form.

| Temperature C° | Native value mm (1/200) | | | Denaturated value mm (1/200) | | |
|-------------------|-------------------------|----------|--------------|------------------------------|----------|--------------|
| | 1st max. | 2nd max. | Ratio (I/II) | 1st max. | 2nd max. | Ratio (I/II) |
| 10 | 20 | 26 | 0.77 | 23 | 46 | 0.50 |
| 20 | 25 | 29 | 0.86 | 31 | 48 | 0.64 |
| 30 | 32 | 35 | 0.91 | 40 | 50 | 0.84 |

Next, the temperature of the medium during the polarographic procedure is a very important factor (Table 2). At higher temperature (30°C) the first maximum is more elevated than the second maximum. This phenomenon was also pointed out by Balle-Helears⁸⁾.

When these factors which have influence on the wave form, are kept strictly constant, it is evident that a certain structure of protein molecule reveals always a consistent protein wave.

Conclusively it can be said that the M_1 wave with prominent first maximum may have high content of conjugated polysaccharide and is supposed to be one of the most interesting fractions on the clinical investigation, whose details will be discussed elsewhere by Yamaguchi.

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