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Simple Elution Procedure for Estimation of the Serum Protein Bands Separated by Disc Electrophoresis in Polyacrylamide Gel *

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Normal sera and sera from patients of several types of cancer were studied by disc electrophoresis in acrylamide gel under conditions close to those of Ornstein and Davis. After staining the proteins with aniline blue black, the main bands were sectioned in slices for a quantitative estimation; the gel was disgregated by stirring with a rod and the protein eluted using a solution made with borate buffer (pH 9.8), acetic acid and sodium lauril sulphate. Remarkable differences were generally observed for the main fractions of cancer sera in comparison with those from normal samples, and also between the respective bands of the various groups of cancer. The three types of haptoglobins (1-1, 2-1 and 2-2) were detected. Conditions for staining glycoproteins with good result are also described.

The high sensitivity and resolution of the method of disc electrophoresis on columns of polyacrylamide gel explain the increasing use of this technique for the study of serum proteins. It detects genetic variants (haptoglobins types 1-1, 2-1 and 2-2) and quantitative changes of the serum proteins. Following the ORNSTEIN (5) and DAVIS (1) method, several authors have described electrophoretic patterns from human normal sera (2, 4, 6, 8) and from patients of various diseases (anemia; infections; liver, renal, neoplastic and neurological diseases) (3, 7, 8, 9).

The existence of a remarkable variety of patterns according to the different diseases is now well establised; as well as a variety of normal patterns which complicate the interpretation of the results. The possibility of using this method not only for the detection of qualitative differences but also for an easy and inexpensive estimation of quantitative changes in the main fractions of the serum samples, without the use of densitometers, was the aim of the present work. We have found a solution which permits a good elution

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of the bands stained by aniline blue black, separated by acrylamide gel in several cases of cancer; it seems that it may be used as a general procedure.

Materials and Methods

SAMPLES. — As a reference, normal sera from healthy humans between 25-30 yrs were used. Pathological sera came from patients of different tumoral affections, arthrosis or tuberculous anexitis. In some cases of cancer, disc electrophoresis of serum proteins was made before and two months later, after telegammatherapy with Co-60.

ELECTROPHORESIS. — The procedure of DAVIS (1) was employed, with the following changes: (*a*) polimerization of the sample gel, 30 min; (*b*) polimerization of the stacking gel, 20 min; this polimerization must be carried out slowly during the first 10 min; (*c*) all polimerizations were performed at a higher temperature than that of the laboratory, with the help of an infrared lamp; (*d*) time for electrophoretic running, 30 min.

STAINING OF PROTEINS. — The gels (removed from the columns) were stained for proteins with 7 % aniline blue black (Acid Black 1) in acetic acid; after elimination of the non-fixed colorant, transparency and visualization in the gels were achieved by immersion in a 7 % acetic acid solution, and generally turning the switch on.

STAINING OF GLYCOPROTEINS. — The detection of glycoproteins was made after electrophoresis by immersion of the gel in 7 % acetic acid solution, at the room temperature during 1 hr, and then in a 0.2 % periodic acid, at 4° C in refrigerator, during 1 hr; finally, it was immersed in the colorant of Schiff, at the temperature of the refrigerator, until apparition of the bands; the decoloration was made with 5 % sodium thiosulphate.

ELUTION OF THE PROTEIN BANDS. — The following solution was used: 0.2 M borate buffer, pH 9.8, 868 ml; 7 % acetic acid, 32 ml; sodium dodecylsulphate, 1 ml; water until, 1 l.

Results and Discussion

For quantitative determinations, elution procedures are, in general, precise and inexpensive; thus, we have employed the following agents for elution of the slices, after staining of the proteins with aniline blue black: 10 N sodium hydroxide, 1 N sulfuric acid, phosphate buffer pH 6.8, borate buffer pH 9.8, 1 % sodium dodecylsulphate in phosphate buffer pH 6.8, 1 % sodium dodecylsulphate in borate buffer pH 9.8, tris-glycine buffer pH 8.9, and 30 % hydrogen peroxide. Although several authors recommend these agents, we have not obtained a satisfactory elution with them. After several assays, we have obtained a good result with the following procedure: The gel, stained with aniline blue black, is sectioned in slices with a shaving blade and the slices are put in centrifuge tubes; then, 2.5 ml of the solution (the composition of which is described in the methods) are added to the tubes; after stirring with a rod during several minutes at the laboratory temperature, the gel is disgregated and the eluate is centrifugated, decanted and measured in a spectrophotometer at 600 nm.

Table I summarizes the quantitative results. The values corresponding to the fractions from normal sera are, in general, similar to those obtained by other authors (2, 4) who employed a densitometer. Remarkable differences between the fractions from cancer sera and normal samples, as well as between the various

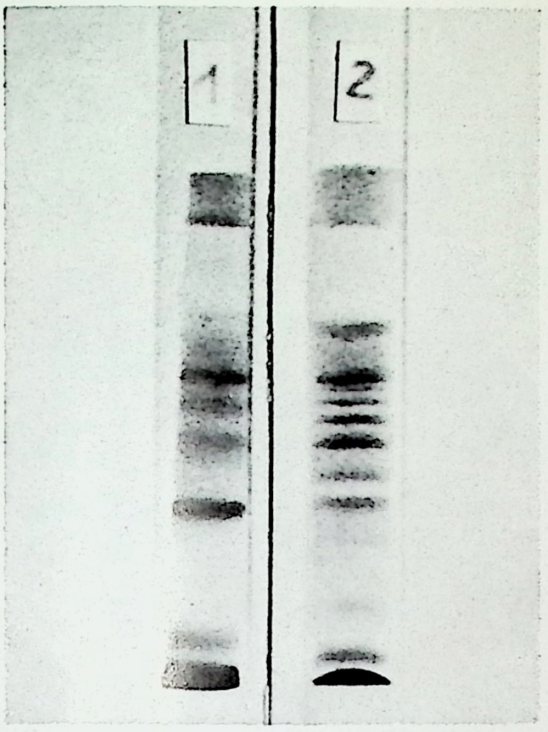

Fig. 1. *Disc electrophoretic patterns of a serum from a patient of lung cancer, before (1) and after (2) telegammatherapy treatment.*

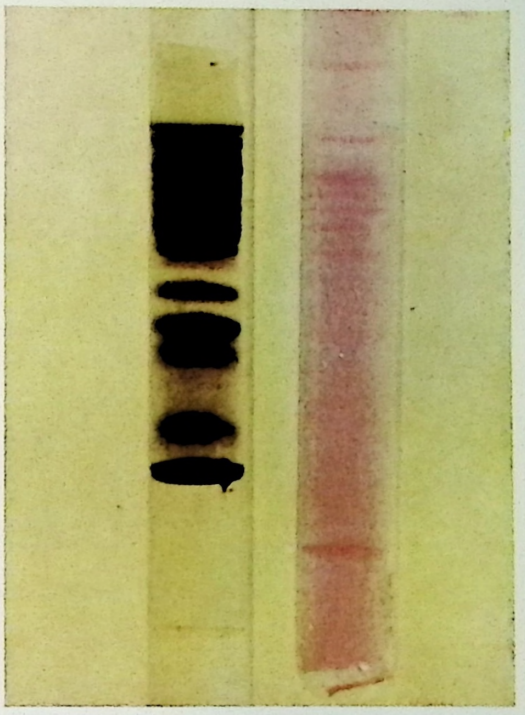

Fig. 3. *Comparison between proteins (left) and glycoproteins (right) of the same serum from a patient of larynx cancer.*

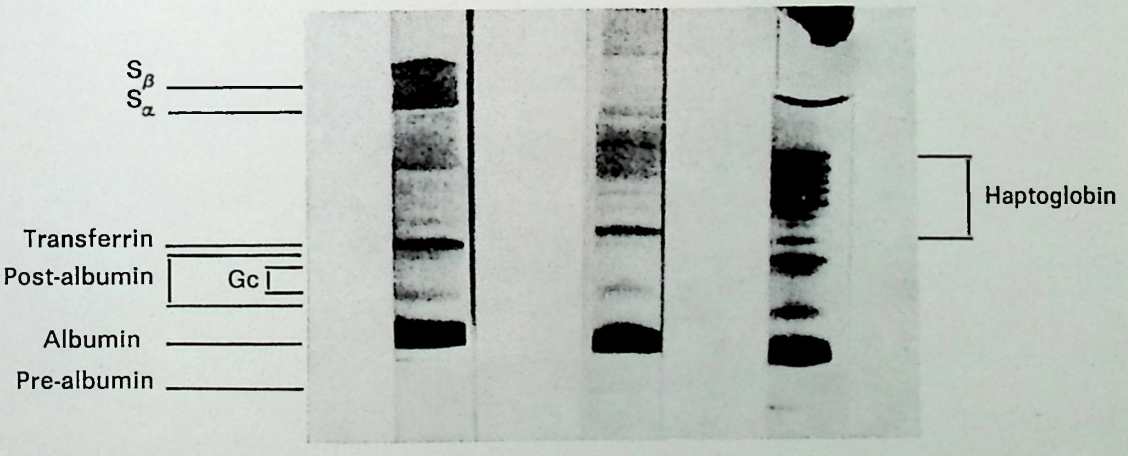

Fig. 2. *Disc electrophoretic patterns of normal adult serum.*
From left to right: Haptoglobins types: 1-1, 2-1 and 2-2. S_α = Slow alpha globulin; S_β = Slow beta globulin; G_c = Group-specific components.

Table I. *Percentage (mean value) of the main serum protein bands after elution from the acrylamide gel.*

(Number of cases between parenthesis)

Samples	FRACTIONS (*)						
	S_α	S_β	Haptoglobin	Transferrin	Post-albumin	Albumin	Preal bumin
Normal (3)	4.40	6.50	11.80	7.90	5.68	63.10	0.45
Cancer (9):							
Lung (3)	9.23	7.09	16.34	3.54	7.52	56.28	—
Bladder (1)	1.26	2.53	8.28	1.30	3.16	83.53	—
Larynx (2)	5.92	4.69	9.80	6.44	4.50	68.64	—
Kidney (1)	1.30	2.60	9.74	1.30	4.54	80.52	—
Mouth ground (1)	2.95	4.05	11.43	3.32	4.45	73.80	—
Uterus (1)	4.80	4.30	13.40	2.87	7.65	66.93	—
Arthrosis (6)	3.49	4.49	8.39	4.20	6.11	75.47	—
Tuberculosis anexitis (1)	2.46	7.39	4.93	1.48	2.95	80.79	—

(*) S_α = Slow alpha globulin; S_β = Slow beta globulin. (See more details in Fig. 2.)

types of cancer, can be observed; it is also true for anexitis and arthrosis cases.

Figure 1 shows the pattern of a serum, from the same patient, before and after telegammatherapy. New bands appeared after treatment.

The three haptoglobin types 1-1, 2-1 and 2-2 were detected. Figure 2 shows the patterns of normal samples. They are similar to those obtained by PASTEWKA *et al.* (6).

We have observed that power supply to eliminate the excess of periodic acid, or for decoloration of the gels, in glycoprotein electrophoresis, is not convenient; very often the bands moved. With our experimental conditions (see Methods) this inconvenient is avoided. Figure 3 permits the comparison between proteins and glycoproteins fractionation corresponding to the same pathological serum.

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