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Staining Technique in Disc Electrophoresis for Disclosing Absence of Normal Serum Proteins in Patients with Hematologic Neoplasms (33825)

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At least 25 protein bands can normally be identified by disc electrophoresis in human serum on polyacrylamide gels stained with aniline blue black. Other reagents are available to aid in the detection of suspect bands. Coomassie brilliant blue R-250 is such a reagent, and is therefore suitable for ascertaining the absence of normal serum proteins in patients with various diseases, such as multiple myeloma.

Materials and Methods. Fifty-five serum samples were collected from patients with various neoplaams. Included were 14 lymphosarcomas (LSA), 11 chronic lymphocytic leukemias (CLL), 12 chronic granulocytic leukemias (CGL), 6 undifferentiated lymphomas (L), 4 multiple myelomas (MM), 4 Hodgkin's disease (HD), 3 acute myeloblastic leukemias (AML), 2 giant follicular lymphomas (GFL).

Twenty-nine specimens obtained from blood donors served as controls. Sera were stored at —20° during the collection period (about 18 months) and clarified immediately before use by centrifugation at 20,000*g* for 20 min.

Disc electrophoresis was performed by the modified method of Ornstein and Davis (1). The anionic gel system provided a final concentration of 7.5% acrylamide with a running pH of 9.3.

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TABLE I. Normal Serum Proteins Absent from Patient Samples.

Patient group[a]	No band X	No band Y
LSA	5	1
CGL	4	3
CLL	3	2
L	2	1
GFL	1	1
MM		1
AML	0	2
HD	0	4

[a] LSA = lymphosarcoma; CLL = chronic lymphocytic leukemia; CGL = chronic granulocytic leukemia; L = undifferentiated lymphoma; MM = multiple myeloma; HD = Hodgkin's disease; AML = acute myeloblastic leukemia; GFL = giant follicular lymphoma.

Three reagents were used to stain protein bands: 0.02% aniline blue black in 3% acetic acid, 0.004% nigrosin in 3% acetic acid, and 0.02% Coomassie brilliant blue R-250 in 7% acetic acid. Gels immersed overnight in each reagent and destained daily with 7% acetic acid for 3 days.

Test preparations of partly purified elastase (20 mg/ml) demonstrate three bands with aniline blue black staining whereas Coomassie brilliant blue R-250 discloses five additional well defined bands without dye precipitation on the external gel surface.

Results. Table I carries the results of this study. One of two separate bands, present in all control sera, was absent in 30 of the 55 sera obtained from patients. Both bands were

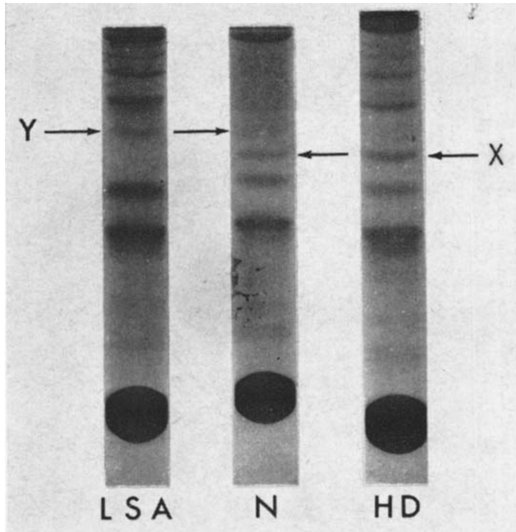

FIG. 1. Polyacrylamide disc electrophoresis of human serum stained with Coomassie brilliant blue R-250. Normal (N) sample shows band (X), which is missing from the lymphosarcoma (LSA) sample, and band (Y), missing from the Hodgkin's disease (HD) sample.

never missing in any one patient. Band X was missing in 36% of the lymphosarcoma sera examined. Band Y was missing in all the Hodgkin's disease samples.

Clarke (2) has proposed an identification system for serum protein bands based on patterns obtained with combined paper and disc electrophoresis. The patterns suggest that X and Y are alpha-2-globulins. This designation agrees with patterns obtained from alcohol fractionated human serum proteins in our laboratory.

The mobility of band X (and band Y) is visually present in Fig. 1. Examples of aniline blue black and nigrosin staining are illustrated in Fig. 2; the Y band is not evident. Such samples frequently demonstrated trace Y bands when stained with Coomassie brilliant blue R-250.

Discussion.

1. Three explanations are possible for the absence of band X or Y in certain sera: (a) the absence of synthesis; (b) partial synthesis of the two protein bands X and Y; or (c) changes in the structure of X or Y, causing the bands to occupy a new position in the gel patterns.

The first and second explanations, absence of synthesis and a partial synthesis, if valid, would represent defects in regulation. The third possibility, changes in the structure of X or Y, could most readily be explained by a mutation in the structural genes coding for X and Y.

2. Proteins X and Y were evident in every control serum, suggesting that their function is necessary for human survival. Ae regulatory defect resulting in an absence of synthesis, therefore, is unlikely for either X or Y.

3. The meaning of the absence of ban X or Y may lie in structural alteration of X or Y, thus influencing its mobility by differences in molecular size, net charge, or conformational stability. Denaturation could prevent the entry of modified X or Y into the separation gel.

4. Preparative disc electrophoresis to iso-

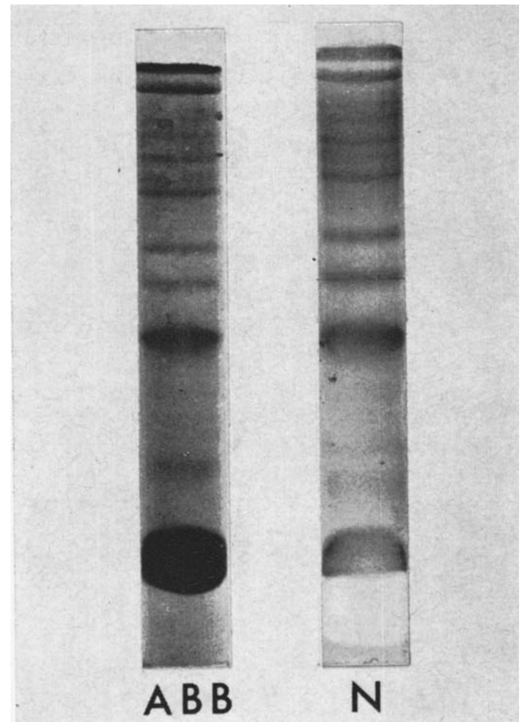

FIG. 2. Polyacrylamide disc electrophoresis of human serum stained with aniline blue black (ABB) and nigrosin (N). Band Y is missing in these patterns.

late X and Y from control sera may resolve the nature of the defects. The technique (3) of preparative disc electrophoresis utilizes a long migration path (22 cm); thus, the two proteins could be obtained in reasonably pure form. Antiserum prepared against X or Y could then be used to test for homologous antigen in patient whole sera. Immunologic assay is sensitive and might detect microgram quantities of either protein, depending on antigenicity. Structurally modified X and Y should be revealed by their cross-reactivity. The location of these proteins will be pinpointed by immunodiffusion of disc resolved samples (4), provided they do migrate in polyacrylamide gel.

Further clarification of these apparent defects will be sought by collecting additional LSA and HD sera for examination. Trends observed within patient groups suggest that differential diagnosis of lymphomas could be expanded with this technique. The size of group samples in the present study precludes extensive correlation at this time.

5. Normal bands do not rule out a prelymphomatous disease.

Summary. The serum protein patterns of 55 patients with various neoplasms were compared by specially stained disc electrophoresis with patterns obtained from 29 control sera. Two control bands, representing at least two different proteins with alpha-2-globulin mobility, were absent from 30 patient sera but were present in all controls. The significance of the differences lies in three possibilities: (i) absence of synthesis; (ii) partial synthesis of the bands, both of which would mean defects in regulation (though this is unlikely); or (iii) altered mobility of the bands, suggesting mutation in the structural genes. The preparative disc electrophoresis technique may resolve the nature of the defect by providing the two proteins in reasonably pure form. Trends in patient groups suggest that possibility of expanded differential diagnosis of lymphomas with this technique.

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