TWO-DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS OF ACID EXTRACTABLE NUCLEAR PROTEINS OF NORMAL RAT LIVER AND NOVIKOFF HEPATOMA ASCITES CELLS

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SUMMARY

The nuclear proteins of normal rat liver and Novikoff hepatoma nuclei were extracted with 0.4 N H_2SO_4 and subjected to two-dimensional polyacrylamide gel electrophoresis. A total of 98 protein components were found in the liver extract and 111 components in the tumor extract. A comparison of the patterns obtained revealed 11 qualitative differences and 5 quantitative differences.

INTRODUCTION

The need to make valid and definitive comparisons for the nuclear proteins of the normal and tumor cell nucleus is suggested by numerous recent investigations where tissue and species specificity have been reported for the nuclear proteins (1-6).

Since one of the key problems is defining the nuclear proteins, the nuclei used in this study were obtained from normal rat liver and Novikoff hepatoma ascites cells by the citric acid method (7-8) which provides nuclei devoid of the outer layer of the nuclear envelope and associated perinuclear contamination. Extraction of protein with dilute H_2SO_4 has been used in studies on histones (9,10), chromatin (11), and nucleolar proteins (10,12).

The two-dimensional polyacrylamide gel electrophoresis technique developed for the separation of ribosomal proteins

(13-15) and adapted to the resolution of nucleolar proteins (12) has enhanced the resolving capability for protein comparisons of tissue components. This electrophoresis technique was applied to the tumor and liver extracts.

Although there are similarities in the proteins of the liver and tumor nuclear acid extracts, several components were found reproducibly in the liver that were not present in the tumor and the opposite was also true. A number of pronounced quantitative differences were also found.

MATERIALS AND METHODS

Preparation of Liver and Tumor Nuclei - Normal male rats were fasted 24 hours prior to sacrifice by decapitation. The livers were perfused with a 0.13 M NaCl, 0.005 M KCl, and 0.008 M $MgCl_2$ (NKM) solution (16) and the distal portions of the lobes were passed through a tissue press.

Novikoff hepatoma ascites cells were obtained by drainage of the ascites fluid after abdominal incision six days after intraperitoneal implantation of the tumors in male rats. The ascites cells were filtered through cheesecloth and washed several times with NKM solution. The cells were collected by centrifugation at 1100 g for 15 minutes.

Nuclei from normal rat liver pressate and Novikoff hepatoma ascites cells were prepared by the 0.025 M citric acid method, pH 2.5 (7,8), using a Tekmar SD-45K Super Dispax system for cell disruption. Nuclei were cleaned by centrifugation through 2.2 M sucrose at 53,000 g for 60 minutes. A final purification was effected by layering the nuclei in one volume of 0.33 M sucrose over two volumes of 0.88 M sucrose and centrifugation at 1100 g for 15 minutes. All extractions were done immediately to avoid variables introduced by freezing or storage.

Sulfuric Acid Extraction of Nuclei - Nuclei were acid extracted two times at 4° by homogenization with a 1:10 ratio of packed nuclear volume to 0.4 N H_2SO_4 volume. The acid extract was cleared by centrifugation at 15,000 g for 20 minutes. Diisopropylfluorophosphate (DFP) was added to the supernatant in a ratio of 0.10 ml of DFP per 25 ml of acid extract, which was then incubated at 4° for one hour. Acid extracts were dialyzed in EDTA-treated dialysis tubing for a total of 12 hours against four changes of 0.01 N HCl and a total of 12 hours against four changes of deionized water. The dialyzed solutions were lyophilized to dryness and stored as dry powders.

Two-Dimensional Acrylamide Gel Electrophoresis - Samples were dissolved at a concentration of 10 mg of protein/1.0 ml in a solution containing 10 M urea, 0.9 N acetic acid and 1% β-mercaptoethanol. Samples were routinely dissolved 18-24 hours in advance of electrophoresis.

Two-dimensional polyacrylamide gels were prepared and run by the method of Orrick et al (12). The first dimensional gel (9.5 cm x 0.6 cm) had the following components: 10% acrylamide, 0.35% bisacrylamide, 0.1% persulfate, 1% TEMED (N,N,N',N-tetramethylethylenediamine) and 4.5 M urea. After 4 hours of preelectrophoresis at 120 volts in 4.5 M urea containing 0.9 N acetic acid, 500 μg of samples were applied and electrophoresed at 120 volts for 6.3 hours at 22° in a fresh electrophoresis buffer containing 0.9 N acetic acid and 4.5 M urea.

The gels were extruded and bisected longitudinally with a taut wire. Half of each gel (250 μg of protein) was adapted for 60 minutes at 45° to 2% sodium dodecyl sulfate (SDS), 6 M urea, 5 mM dithiothreitol (DTT) and 0.1 M phosphate (pH 7.1). After an additional hour of adaptation to 0.1% SDS, 6 M urea, 5 mM

DTT, and 0.01 M phosphate (pH 7.1), the gel slice was placed on the 10 cm side of a 10 x 9.5 x 0.3 cm slab gel which had the following components: 12% acrylamide, 0.31% bisacrylamide, 0.05% TEMED, 0.1% SDS, 0.075% persulfate, 6 M urea, and 0.1 M phosphate buffer (pH 7.1). The first dimensional gel was polymerized in a cap gel of the same concentration as the slab except for 0.01 M phosphate (pH 7.1). The slab gels were run for 14 hours at 50 mA/slab in an electrophoresis buffer of 0.1% SDS and 0.1 M phosphate (pH 7.1) at 22°. The slabs were extruded and stained for 4 hours in 0.25% Coomassie brilliant blue R in methanol-acetic acid-water (10:2:10). Slabs were destained in many changes of methanol-acetic acid-water (1:2:17).

RESULTS

Two-Dimensional Electrophoretic Separation of the 0.4 N H_2SO_4 Extract of Normal Rat Liver Nuclei - Nuclear acid extracts were prepared from the citric acid nuclei of normal rats. Two-dimensional polyacrylamide gel electrophoresis of the liver extract provided the pattern shown in Figure 1a. There were 98 different protein components counted (Fig. 1a). A drawing of the liver acid extract pattern was prepared from the examination of duplicate gels from the same sample and from gels originating from different nuclear preparations. The composite is shown in Figure 1b. The pattern was divided into three regions (A, B and C) and each spot was given a number relating to the identical migration of a known nucleolar spot (12) or a lower case letter for a spot not found in a nucleolar pattern. There were 38 spots that were not previously observed in the normal rat liver nucleolar acid extract pattern (12).

Certain spots were identified with respect to the migration of known proteins. The spots GAR, A1 and A4 had the same mo-

bilities as the GAR (f_{2a1}), AL (f_{2a2}) and AR (f_3) histones (17,18), as determined by the positions of the corresponding highly purified histones run individually on two-dimensional gels.

Two-Dimensional Electrophoretic Separation of the 0.4 N H_2SO_4 Extract of Novikoff Hepatoma Nuclei - The acid extract from Novikoff hepatoma citric acid nuclei was prepared in the same manner as the extract from liver nuclei. A two-dimensional polyacrylamide gel for the tumor extract is shown in Figure 2a. There were 111 components counted on this gel (Fig. 2a). A composite drawing was prepared for this extract from duplicate gels and preparations. The composite is shown in Figure 2b. In the case of the tumor there were 45 spots which were not previously found in the Novikoff nucleolar acid extract pattern (12).

The tumor pattern was divided into three regions (A, B and C). The marker spots used for comparison with the liver pattern were A1-4, A17-19, A24-25, B7, B13, B23-25, B33-34, C2, C13 and C23-24. The proteins which migrated like histones in the liver pattern could also be identified in the tumor pattern.

Comparison of the Two-Dimensional Patterns Obtained from Liver and Tumor - A comparison of the composites drawn from the liver and tumor patterns (Fig. 1b and 2b) indicated that most of the acid extractable proteins of the normal liver nucleus were also in the hepatoma extract. There were 84 spots common to the two patterns.

A few major qualitative differences were consistently observed. They are shown in Table I. Spots B2, B5L, Bp, Bt and Cf were found in the liver. Spots A10, Ao, B5, Bs, C18 and Cj were found in the tumor. The A10 spot was shown to be of greater

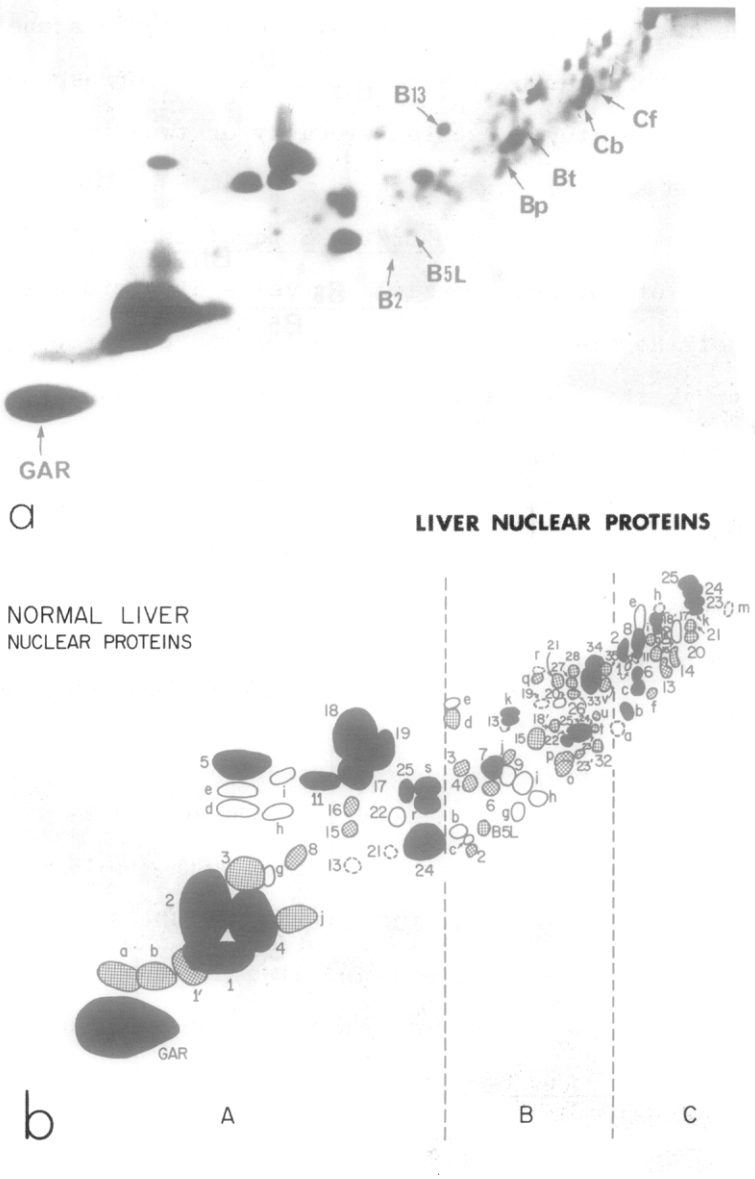
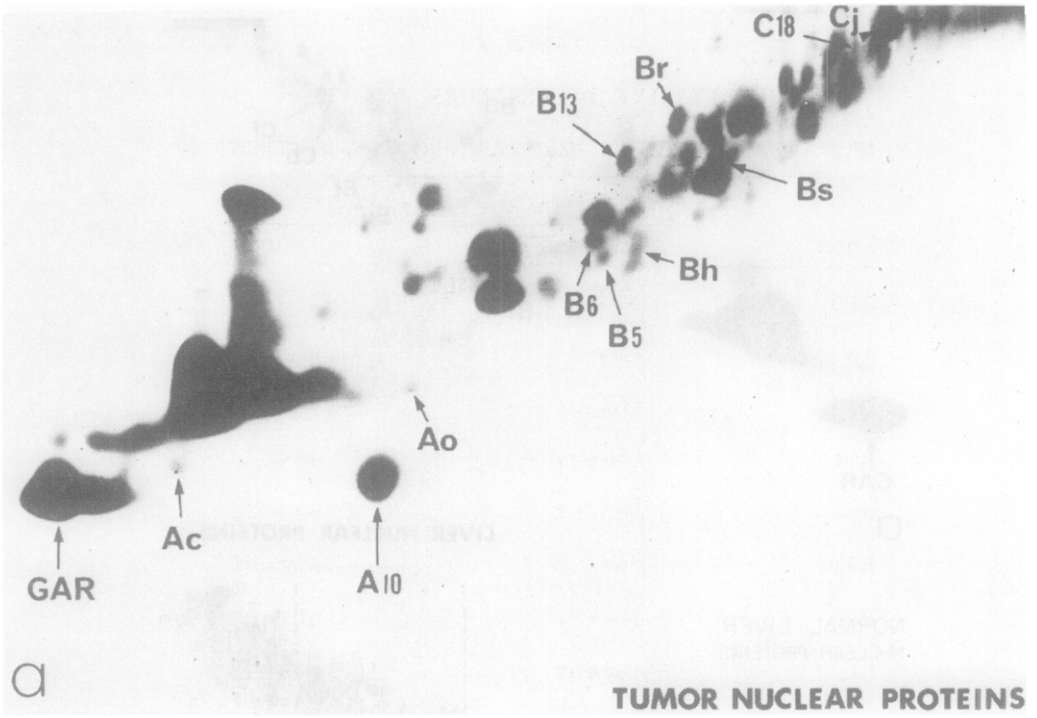


Fig. 1a Two-dimensional polyacrylamide gel electrophoresis of 250 μg of normal rat liver nuclear proteins. Samples were run in the first dimension on tube gels of 10% acrylamide, 6 M urea, 0.9 N acetic acid at 120 V for 6.3 hrs. For the second dimension, a 12% acrylamide, 0.1% SDS slab gel was run for 14 hrs at 50 mA/ slab. Gels were stained with Coomassie brilliant blue R.

Fig. 1b Diagrammatic representation of the electrophoretic pattern of Figure 1a. The most dense and large spots are black, the less dense spots are cross-hatched, the even less dense spots are open circles, and minor spots are broken circles.



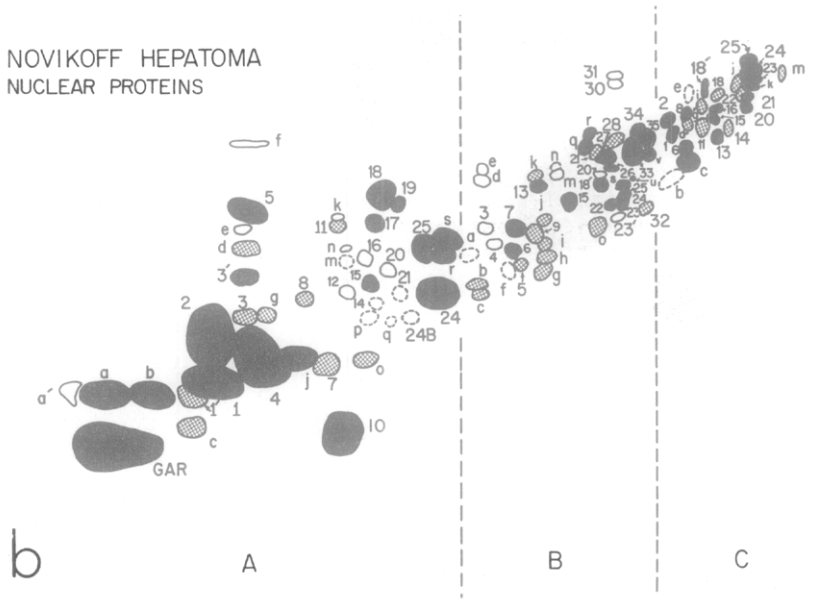


Fig. 2a Two-dimensional polyacrylamide gel electrophoresis of 250 μg of Novikoff hepatoma nuclear proteins. See Figure 1a for conditions.

Fig. 2b Diagrammatic representation of the electrophoretic pattern of Figure 2a. See Figure 1b for legend.

TABLE I

QUALITATIVE DIFFERENCES FOR THE

HEPATOMA AND LIVER NUCLEAR PROTEIN PATTERNS

	Density of Spot	
Spot	Hepatoma	Liver
A10	++++	Absent
Ao	+++	Absent
B2	Absent	+++
B5L	Absent	+++
Bp	Absent	+++
B5	+++	Absent
Bs	++++	Absent
Bt	Absent	++++
Cf	Absent	+++
C18	+++	Absent
Cj	+++	Absent

Tabulation of the qualitative differences between normal rat liver and Novikoff hepatoma nuclear protein patterns. Only the cross-hatched or solid spots were included in this comparison.

intensity in Novikoff nucleolar acid extracts (12). Spots A17, A18 and A19 (Fig. 1b,2b), which are the very lysine-rich (f_1) histones on the basis of their migration in the first dimension (19,20), were of similar intensity but were consistently larger in the liver pattern. A number of striking quantitative differences are shown in Table II. In the liver pattern, Spot Cb had a greater intensity. Spots Ac, B6, Bh and Br were more pronounced in the hepatoma.

DISCUSSION

A rapid and simple method for nuclear isolation and ex-

TABLE II

QUANTITATIVE DIFFERENCES FOR HEPATOMA AND LIVER NUCLEAR PROTEIN PATTERNS

Spot	Density of Spot	
	Hepatoma	Liver
Ac	+++	< +
B6	++++	< +
Bh	+++	+
Br	++++	+
Cb	+	++++

Tabulation of the quantitative differences between normal rat liver and Novikoff hepatoma nuclear protein patterns.

traction has been applied to normal rat liver cells and Novikoff hepatoma ascites cells. This method releases many proteins from the nucleus, maintains the solubility of the acid-extractable components, and lends itself to the survey of tumor and normal tissue nuclear protein comparisons. The acidic conditions maintained during the nuclear isolation and extraction minimize the probability of proteolytic degradation in this study.

The extracts were compared by two-dimensional polyacrylamide gel electrophoresis and found to have 98 components in the case of the liver and 111 components in the case of the tumor. The similarity between liver and tumor gel patterns was very striking. Most of the acid extractable proteins of the normal rat liver nucleus are common to the Novikoff hepatoma nucleus as evidenced by the 84 common spots between the two gel patterns.

A few major qualitative and quantitative differences were observed. Spots B2, B5L, Bp, Bt and Cf were found in the liver

while spots A10, Ao, B5, Bs, C18 and Cj were found in the tumor. While spot A10 was much larger in tumor nucleolar extracts, spot B5L was only found in the liver nucleolar extracts (12). Spot Cb was found at higher levels in the liver while spots Ac, B6, Bh and Br were more intense in the tumor.

The reasons for the differences mentioned may involve the presence or absence of control proteins, differences in the modification of the same proteins from liver to tumor or the result of the specific increases in nuclear metabolism in tumor cells. These studies are now being extended to other growing tissues, including regenerating liver and other tumors.

The usefulness of the two-dimensional fingerprinting method for nucleolar proteins is now demonstrated by these studies on nuclear extracts. This approach to comparison of normal and tumor cell nuclear proteins may be useful in screening for differences in a number of pathological states.

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