ELECTROPHORETIC FRACTIONATION OF PROTEINS OF NUCLEAR MEMBRANES OF THE RAT LIVER AND HEPATOMA

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A number of protein fractions were detected by electrophoresis in acid polyacrylamide gel in nuclear membranes, some of them closely resembling histones in their mobility. The electrophoretic pattern of the two fractions of nuclear membranes was not identical. Hepatoma nuclear membranes differ in their electrophoretic pattern from those of normal liver.

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Earlier papers [1, 3] described the preparation of isolated nuclear membranes of certain normal and neoplastic animal cells. Two fractions of nuclear membranes from rat liver with floating densities of 1.19 and 1.16, respectively, differed both from each other and from other cell membranes, not only in their composition, but also in their enzymic activity [2, 4]. One difference was that the "heavy" fraction contained twice as much protein per dry weight (about 54%) and the "light" fraction (about 27%). It was postulated on the basis of these results that the "heavy" fraction (ρ 1.19) corresponds to the inner (true) nuclear membranes, while the "light" fraction (ρ 1.16) is derived from the outer nuclear membrane [3].

In the present investigation proteins of both fractions of nuclear membranes of the rat liver and hepatoma were subjected to electrophoresis on polyacrylamide gel parallel with total nuclear proteins of rat liver and total histones of calf thymus.

EXPERIMENTAL METHOD

Nuclei were obtained from the rat liver by Hiatt's method [6], and from tumor cells by Harris's method [7]. Nuclear membranes were isolated by a method developed by ourselves.

Electrophoresis of the proteins was carried out by the usual method at pH 8.3 [5], and by the same method in Neville's modification [8] suggested for electrophoresis of the proteins of plasma membranes, at pH 4.0 and 2.7. The sample of membranes containing 200-300 μg protein was treated with (in final concentration) K_2CO_3 (0.05 M), urea (8 M), mercaptoethanol (10%), sucrose (20%), and a few crystals of methyl green. Part of the protein, probably corresponding to structural proteins of the membranes, remained at the starting line.

The specimen thus obtained was applied to 2.3% acrylamide gel (Cyanogum-41, pH 5.9), containing 6 M urea solution. In the top buffer (pH 4.0) the final urea concentration was 6 M. No urea was added to the lower buffer, pH 2.7. The current applied to each tube was 0.75 mA. On the arrival of protein in the lower gel the current was increased to 1.5 mA per tube. Electrophoresis continued for 2-3 h. After its end the gel was stained with 0.5% amido black, the excess of which was washed off with 7% acetic acid solution in the usual manner.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1b that liver nuclear proteins were separated into 21 fractions. Depending on their mobility, the protein fractions could be placed in four groups (starting with the least mobile):

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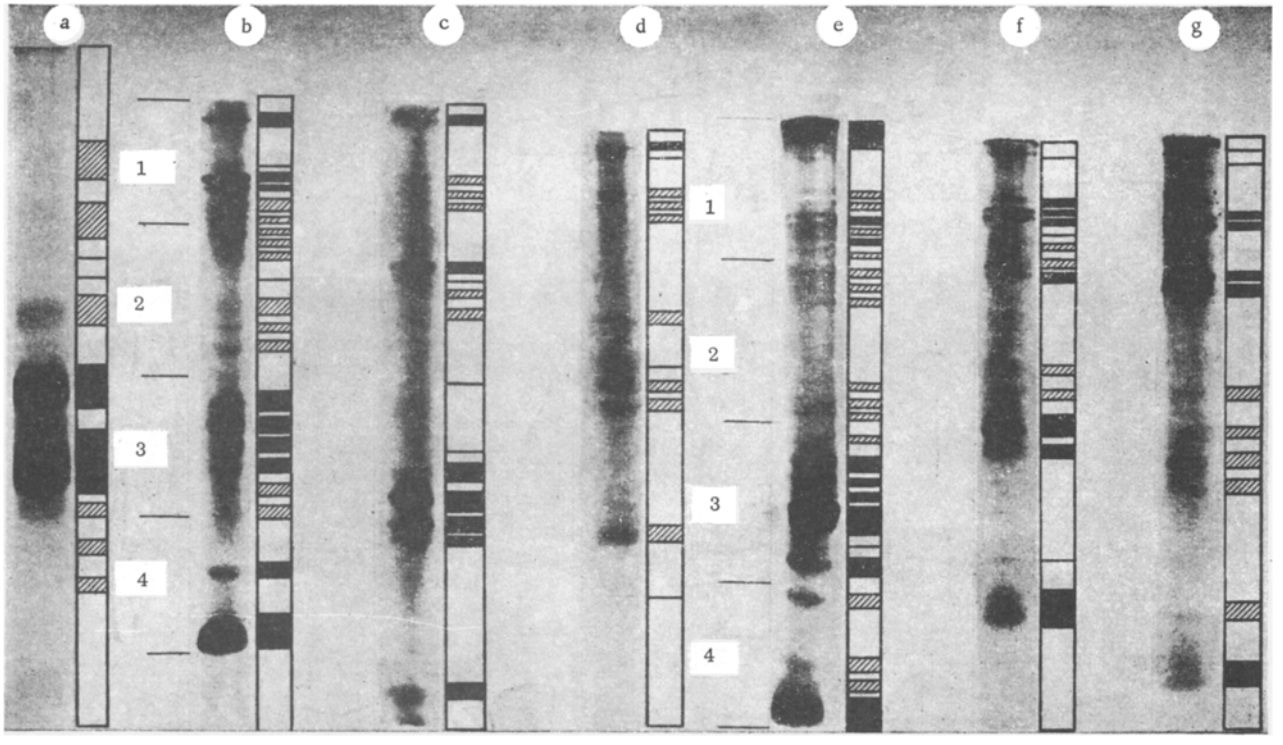

Fig. 1. Electrophoresis of proteins from nuclei and nuclear membranes of rat liver and hepatoma in polyacrylamide gel. a) Calf thymus histones; b) liver nuclear proteins; c) nuclear membrane proteins of liver (ρ 1.19); d) nuclear membrane proteins of liver (ρ 1.16); e) hepatoma nuclear proteins; f) nuclear membrane proteins (ρ 1.19) of hepatoma; g) nuclear membrane proteins (ρ 1.16) of hepatoma. 1, 2, 3, 4) Group numbers denoting differences in mobility of protein fractions.

group 1, slowly moving components; groups 2 and 3, fractions with increasing mobility; and group 4, protein fractions moving rapidly toward the cathode.

Comparison of the electrophoretic behavior of total nuclear proteins and histones (Fig. 1a) showed that in their mobility the nuclear proteins of group 3 corresponded to the most strongly stained, rapidly moving histone fractions.

Electrophoresis of proteins of the "heavy" membrane fraction (ρ 1.19) of nuclear membranes of the liver cells revealed 15 fractions (Fig. 1c), and like the proteins of total nuclei, these could be divided also into four groups. However, in the case of the nuclear membranes these groups differed not only in the number of their components, but also in the relative intensity of their staining. The components of group 3, corresponding to the most strongly stained fractions of histone from calf thymus, were most clearly revealed by electrophoresis.

During electrophoresis of proteins of the "light" nuclear membranes of the liver the quantity of protein applied did not exceed 100 μg. Nevertheless, even with this small amount of protein applied to the starting line, 11 fractions were discovered (Fig. 1d). Only one of these fractions corresponded to the histone fractions. Total hepatoma nuclear proteins separated during electrophoresis into 23 fractions (Fig. 1e). Conventionally these could also be divided into 4 groups, similar to those of the rat liver nuclei.

Proteins of the "heavy" fraction of hepatoma nuclear membranes separated into 14 fractions (Fig. 1f), and proteins of the "light" fraction into 12 components (Fig. 1g). There was comparatively little difference between the electrophoretic behavior of proteins of the "light" and "heavy" fractions of the hepatoma nuclear membranes.

Hence, by electrophoresis of proteins from nuclei and nuclear membranes in acid polyacrylamide gel between 21 and 23 fractions were discovered for the nuclear proteins and from 11 to 15 components for the "light" and "heavy" fractions of the nuclear membranes. During electrophoresis in alkaline polyacrylamide gel, separation of the membrane proteins into fractions was less successful: in the case of proteins of "heavy" nuclear membranes of the liver only 7 fractions could be obtained.

During electrophoresis in acid gel (pH 2.7, pH 4.0) not only heterogeneity of the nuclear membrane proteins (ρ 1.19 and ρ 1.16) of the rat liver was demonstrated, but differences were also found in the mobility and intensity of staining of the protein fractions.

It is a general rule that nuclear membranes contain components which correspond in their mobility to the principal fractions of total histone. Whereas no sharp differences were found between the electrophoretic mobilities of proteins of tumor and normal nuclei, the proteins of the nuclear membranes from normal and tumor cells differed in their behavior.

Qualitative and quantitative differences in the protein composition of the two fractions (ρ 1.19 and ρ 1.16) of the liver nuclear membranes are in agreement with results showing differences in their enzyme systems [3, 4].

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