ELECTROPHORETIC COMPARISON OF NUCLEAR AND NUCLEOLAR PROTEINS

I. BEEF PANCREAS

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(Received May 25th, 1960)

SUMMARY

Nuclei and nucleoli, isolated from beef pancreas, are extracted with 0.14 *M* NaCl, 1 *M* NaCl and 0.1 *N* NaOH in the order given. The 0.14 *M* NaCl and 0.1 *N* NaOH extracts lend themselves to paper and agar electrophoresis and the electropherograms obtained from these extracts of nuclei and nucleoli are compared. It is shown that the proteins of the nucleoli are in several aspects different from the proteins of the non-nucleolar part of the nucleus.

INTRODUCTION

Apart from histones and enzymes little work has been done on the proteins of the nucleus[1,2]. DALLAM[3] has tried to give an inventory of all proteins present, KIRKHAM AND THOMAS[4] have purified one protein component extracted with 0.14 *M* NaCl and WANG *et al.*[5] a protein extracted with 0.1 *N* NaOH.

Only one group of workers, viz. MONTY *et al.*[6], have isolated nucleoli from mammalian tissue and they have restricted themselves to the determination of the histone content.

We will describe the isolation of nuclei and nucleoli from beef pancreas and the study by means of electrophoresis of the protein mixtures extracted from them.

MATERIALS AND METHODS

As soon as possible after the death of the animal, the pancreas was transported on ice to the laboratory. All isolation and extraction procedures described below were carried out at 1–3°.

Isolation of nuclei

By a method described previously[7], pure nuclei were obtained (Fig. 1). They were free from cytoplasmic adhesions as was shown by electron microscopy (Fig. 2).

Isolation of nucleoli

In our hands the preparation of nucleoli with the help of sonic vibration according to MONTY *et al.*[6] proved to be too destructive. The larger nucleoli were destroyed, only

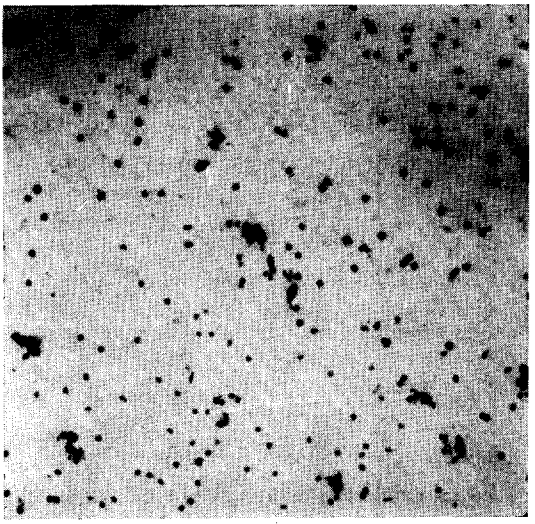

Fig. 1. Isolated nuclei from beef pancreas. Objective 10×.

Fig. 2. Electron micrograph of an isolated nucleus. (30000 ×).

the smaller ones were left undamaged. It appeared, however, that in the viscous glycerol medium used, undamaged nucleoli could be liberated from nuclei in a small volume homogenizer (Bühler).

The following procedure was applied: nuclei from 800 to 900 g pancreas (± 400 mg) were suspended in 70 % glycerol phosphate, the same medium as used for the isolation of nuclei. This suspension was cooled to —5° and homogenized during 2 min at 40,000 rev./min. The temperature rose to about 0°; this procedure, cooling to —5°, followed by 2 min homogenizing, was repeated until microscopic investigation showed that nearly all nuclei were disrupted. Then the suspension was diluted to a glycerol concentration of 10 %, the phosphate concentration being kept constant. The resulting suspension was centrifuged at 500 × *g* for 5 min. The supernatant was discarded, the sediment resuspended in 100 ml 10 % glycerol phosphate and the suspension left overnight in a high measuring glass. In this medium the whole nuclei and larger nuclear fragments sediment more rapidly than nucleoli. The upper layer was collected by repeatedly pipetting off small samples of the suspension until nuclei began to make their appearance in a sample.

The nucleoli were spun down at 500 × *g* for 5 min and were in general satisfactorily pure (Fig. 3). They were still surrounded by varying amounts of nucleolus associated chromatin. This was established by staining with methylgreen pyronin. The red nucleolar body is seen encircled by the blue green chromatin.

Fig. 3. Isolated nucleoli with in the lower left corner a nucleus at the same magnification.

Extraction of protein

The same solutions have been used as by DALLAM[3], viz. 0.14 *M* NaCl, 1 *M* NaCl and diluted NaOH in the order given.

For a complete extraction of the proteins soluble in 0.14 *M* NaCl, it is necessary that the nuclei are completely disrupted. When intact nuclei are suspended in this solution, only 20 % of the 0.14 *M* NaCl soluble proteins are extracted*. We have

* This finding gives reason to doubt that the isolation of nuclei in aqueous media causes such great losses in protein as is stated by several authors. These authors used the nuclei isolated by the BEHRENS method as a standard of comparison, but nuclei isolated in aqueous media should not be compared to nuclei obtained by the BEHRENS method, as the latter in reality are nuclear fragments, not any longer protected against the medium by a membrane.

achieved destruction of the nuclei either mechanically by homogenizing or by addition of a detergent.

The isolated nuclei were twice washed as quickly as possible with a small quantity of 0.14 *M* NaCl. Then they were suspended in 0.14 *M* NaCl and homogenized during 2 min in a Bühler homogenizer and cooled. In general this procedure, in which the temperature was kept below 5°, had to be repeated 6 times before the greater part of the nuclei was disrupted. Then the suspension was shaken vigorously overnight, while the next morning the homogenizing was repeated for 3 × 2 min. Finally, the suspension was centrifuged at 20,000 × *g* for 10 min. The supernatant is the 0.14 *M* NaCl extract.

The sediment was washed twice with 0.14 *M* NaCl and shaken with 1 *M* NaCl for 6 to 7 h. Centrifuging during 15 min at 20,000 × *g* resulted in a clear supernatant, which is the 1 *M* NaCl extract*. The sediment was washed with 1 *M* NaCl and thereafter shaken overnight with 0.1 *N* NaOH**. The supernatant after 30 min centrifuging at 20,000 × *g* is the 0.1 *N* NaOH extract.

There is a small residue from which no more protein could be extracted. Of the total protein extracted, approx. 30 % is found in the 0.14 *M* NaCl extract, approx. 50 % in the 1 *M* NaCl extract, and about 20 % in the 0.1 *N* NaOH extract.

As we wished to compare the proteins extracted from whole nuclei and from nucleoli, the same solutions were used for the extraction of the nucleoli and as homogenizing had no visible effect on the nucleoli, we thought it advisable to add 0.3 % saponine*** to the 0.14 *M* NaCl solution used in order to obtain complete extraction.

After each extraction step the nucleoli were examined by means of the Feulgen staining of the sediment. Before the extraction the nucleoli were, as stated above, more or less surrounded by Feulgen positive material; after extraction with 0.14 *M* NaCl and saponine the colour caused by the Feulgen staining had much diminished, while 1 *M* NaCl extraction makes the staining very weak. In the almost negligible sediment remaining after treatment with 0.1 *N* NaOH no nucleoli could be distinguished.

Electrophoresis

We applied horizontal paper electrophoresis, using Whatman No. 1 paper and 1/15 *M* phosphate buffer, ph 8.0. The strips were stained with Bromophenol blue.

Our technique for agar electrophoresis is essentially that described by WIEME AND RABAEY[8]; the phosphate buffer was approx. 0.01 *M*, so that the current did not exceed 7–8 mA. The extract was applied in a slit-like depression, made by lowering a metal strip into the agar to 3/4 of the thickness of the layer. The extracts from nuclei and nucleoli respectively were applied side by side on one slide making direct comparison between them possible.

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* The proteins of the 1 *M* NaCl extract were not amenable to the electrophoresis techniques used. The 1 *M* NaCl extraction was nevertheless performed to prevent nucleohistones from appearing in the 0.1 *N* NaOH extract.

** Several attempts at the substitution of the 0.1 *N* NaOH extraction by a milder treatment were unsuccessful: buffers up to ph 11 extracted hardly any protein, even 0.01 *N* NaOH extracted only half the amount obtained by 0.1 *N* NaOH.

*** Saponine in this concentration had been shown not to influence the electrophoretic behaviour of the proteins extracted from whole nuclei.

Absorption measurements

The abosorption spectra were determined with the help of a double monochromator and a stabilized H_2 lamp as a light source. A photomultiplicator tube and a galvanometer were used for the measurements.

RESULTS

0.14 M NaCl extracts

The absorption spectra of the extracts of both nuclei and nucleoli are identical, with a maximum of absorption situated at 265 mμ, suggesting the presence of (poly)-nucleotides.

The paper electropherograms shown in Fig. 4 from extracts with roughly equal protein concentrations are made under identical conditions and demonstrate that the nuclear extract contains at least 6 components, the nucleolar extract at least 5 components, and that the two extracts differ in several aspects from each other.

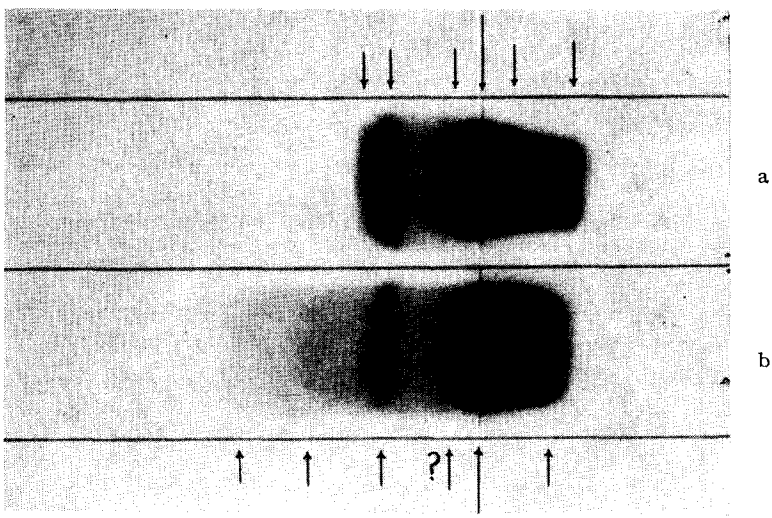

Fig. 4. Paper electropherograms of 0.14 *M* NaCl extract from: a, nuclei; b, nucleoli. 1/15 *M* phosphate buffer p_H 8.0; 5 V/cm; 0.5 mA/cm; 20 h.

Especially at the negative side of the starting line the difference is clearly visible. Of the two closely adjacent components which are found at this side in the nuclear extract, only one is also seen in the extract of the nucleolus. In the latter, however, there are two other components that migrate quickly to the negative electrode. They must be proteins with an iso-electric point considerably higher than the p_H (8.0) of the buffer. At the positive side of the starting place the difference is less spectacular, but nevertheless clear.

These findings could be confirmed by the electrophoresis in agar. Fig. 5 represents the actual densitometric curves of a pair of electropherograms obtained from the nuclear and nucleolar extract respectively. In the curves the peaks and shoulders consistently found in several experiments are indicated by arrows. These electropherograms too are directly comparable, as they were run on the same slide. However,

in these experiments the protein concentration of the nucleolar extract could not be made equal to that of the nuclear extract. All components but one (at the negative side) differ in their electrophoretic behaviour.

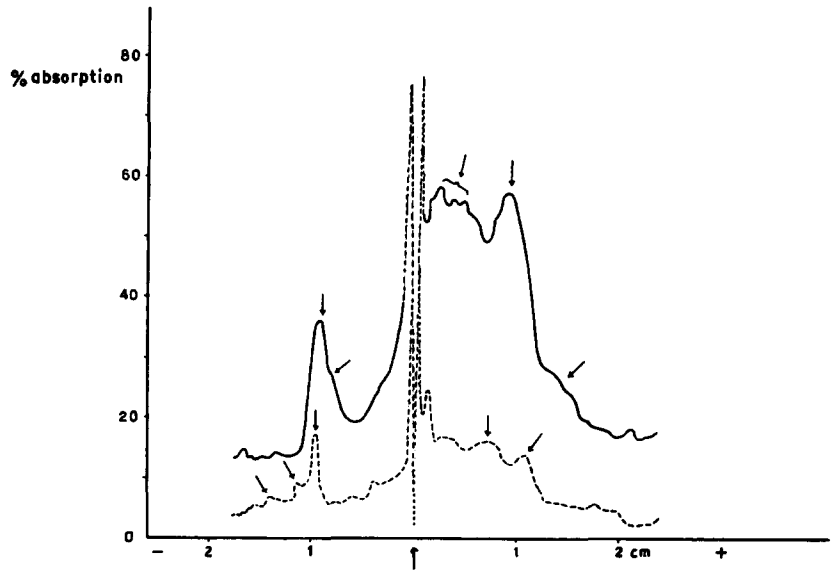

Fig. 5. Densitometric curves of agar electropherograms after staining with Amido Black (transmission of slide assumed to be 100%). 0.14 *M* NaCl extract of nuclei (drawn line) and nucleoli (dotted line). 0.014 *M* phosphate buffer p_H 8.0; 175 V; 7 mA; 15 min.

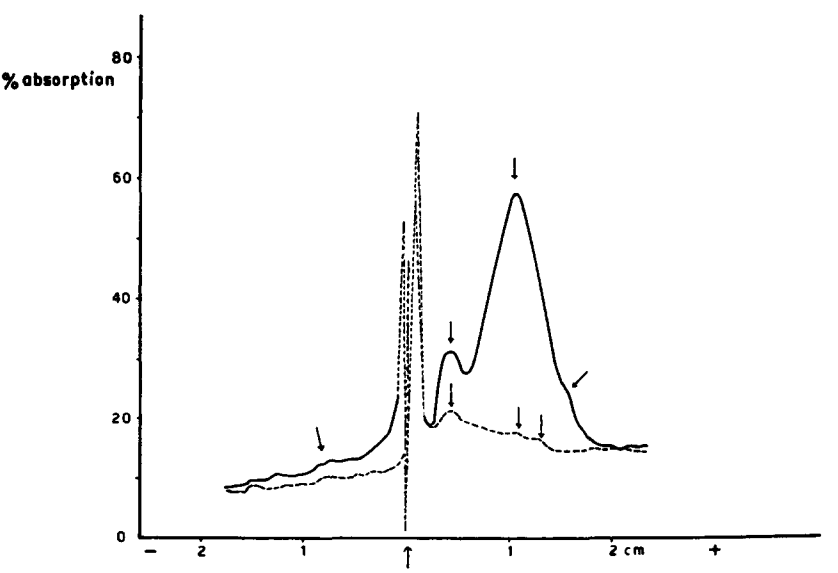

Fig. 6. Densitometric curves of agar electropherograms after staining with Amido Black. 0.1 *N* NaOH extract of nuclei (drawn line) and nucleoli (dotted line). 0.014 *M* phosphate buffer p_H 8.0; 175 V; 7 mA; 15 min.

0.1 N NaOH extracts

The absorption spectra of the extracts of nuclei and nucleoli are identical, and similar to those obtained by DALLAM[3].

In paper electrophoresis of this extract it was necessary to apply the extract far to the side of the negative electrode to force the proteins to migrate, and the electrophoretic pattern was found to be strongly dependent on the concentration of the proteins.

No such influence of the protein concentration was found in agar electrophoresis. Moreover, the resolution obtained by this technique was much better. Fig. 6 shows the result of the electrophoresis of both the nuclear and nucleolar extracts. The consistently found components are again indicated by arrows. Comparison shows that the largest fraction in the nuclear extract is reduced to an insignificant peak in the nucleolar extract, while the component second in importance in the nuclear extract, is the main one in the nucleolar extract. One gets the impression that the differences between the 0.1 *N* NaOH extracts of nuclei and nucleoli are of a quantitative rather than of a qualitative nature.

DISCUSSION

The conclusion that the nucleolar proteins differ from those of the rest of the nuclei, partly in a quantitative, partly in a qualitative sense, is only permitted if the possibility is excluded that non-nuclear proteins, dissolved in the medium during the preparation of the nuclei, penetrate into the nuclei. In order to investigate this point we have prepared a particle-free supernatant of the homogenate from which we used to isolate the nuclei. This supernatant was dialyzed against distilled water, and freeze-dried. All proteins in the supernatant were soluble in 0.14 *M* NaCl. Paper electrophoresis of a solution of appropiate concentration showed that the chief fractions found, the 0.14 *M* NaCl nuclear extract were not found in the supernatant and, conversely, the most conspicuous protein component in the supernatant had no counterpart in the nuclear extract. These findings imply that the proteins extracted from the nuclear preparations do, in fact, come from the nuclei. It can not be said that all proteins obtained by nucleolar extraction originate from the nucleoli. We did not isolate the pure nucleolar body, but these cell particles were always more or less surrounded by the nucleolus-associated chromatin. It is probable that the proteins in the 0.14 *M* NaCl and the 1 *M* NaCl extracts from nucleoli partly represent proteins from the chromatin layer around the nucleolus.

That, nevertheless, the 0.14 *M* NaCl extract from nucleoli is quite different from that from whole nuclei may mean that the nucleolus associated chromatin differs from the non-nucleolus associated chromatin, or else, that in whole nuclei the 0.14 *M* NaCl soluble proteins associated with chromatin, nucleolus associated or not, are completely lost in those contained in the nuclear sap.

In this respect there is less uncertainty as to the origin of the 0.1 *N* NaOH extract of nucleoli, as by the time this extract is prepared the nucleoli have apparently lost their chromatin shells.

ACKNOWLEDGEMENTS

The author wishes to thank Professor M. T. JANSEN for his helpful advice and constructive criticism.

Many thanks are due to Miss H. A. BERTRAM for invaluable technical assistance during the course of the work.

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