THE ISOLATION OF GLOBULINS FROM CELLULAR NUCLEI*

BY WILLIAM R. KIRKHAM† AND LLOYD E. THOMAS

(From the Department of Biochemistry, University of Missouri, Columbia, Missouri)

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Behrens in 1932 (1) developed a method for isolating cellular nuclei. Lyophilized tissue was finely ground and the nuclei were isolated from other cellular débris by repeated centrifugation in organic solvents of suitable densities. The greater density of the nuclei makes this procedure possible. The method has been employed by various workers (2–5). Dounce et al. (4) reported that approximately one-half of the mass of these nuclei was removed by extraction with water adjusted to pH 6.0 with very dilute citric acid. 0.9 per cent sodium chloride solution also extracted much material from the nucleus, indicating that proteins other than nucleohistone and lipoprotein are present in appreciable quantities in nuclei. Nucleoprotein and lipoprotein (6) are predominantly present in nuclei isolated in aqueous media. Results of other investigations (7, 8) suggest that relatively large quantities of protein may be lost from nuclei isolated in aqueous media.

The present paper reports an investigation of the nature of the proteins soluble in dilute salt solutions, proteins which are extractable from nuclei prepared by the Behrens method.

EXPERIMENTAL

Isolation of Nuclei—Nuclei of calf thymus and calf liver† were isolated by a modification of the procedure of Behrens. The procedure employed was somewhat variable with each preparation, and is not presented in detail since it is quite similar to that of Dounce and coworkers (4) and that more recently presented in detail by Allfrey et al. (5). The tissues were lyophilized and finely ground with petroleum ether in a ball mill. The separation of nuclei from other tissue components was carried out by

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‡ The calf thymus and calf liver were generously supplied by the Research Division of Armour and Company. Soon after the animals were killed, they were frozen and packed in solid carbon dioxide.
repeated centrifugation in a medium of benzene-carbon tetrachloride at various specific gravities. Control samples of nuclei were isolated at 4°. Other samples were prepared at room temperature. Satisfactory separation of nuclei (both thymus and liver) from tissue débris was obtained at a specific gravity of 1.350. Flotation of the nuclei was effected at a specific gravity of 1.395. Extreme care was taken in the isolation to insure pure preparations of nuclei. Purity of nuclear preparations was judged by the absence of cytoplasm when the nuclei were stained with one or more of the following: hematoxylin-eosin, methyl green, crystal violet, or acetocarmine. In cases in which purity was doubtful, the nuclear preparations were reground and the isolation procedure repeated.

Extraction of Proteins—All protein extraction and purification were carried out at 1-4°. Samples of 2 to 8 gm. of isolated nuclei were extracted by stirring for a period of 12 to 18 hours with 100 ml. of 0.14 M sodium chloride per gm. of nuclei. After the 12 to 18 hour stirring period, the extraction mixture containing clumped nuclei was homogenized in an Elvehjem-Potter homogenizer, stirred an additional 6 to 8 hours, and centrifuged. The residue was lyophilized. The supernatant was examined for protein constituents.

Determination of Amount of Substances Extracted—Weighted samples of nuclei were carefully extracted as described. The lyophilized residue from each sample was weighed and the amount of material extracted was calculated by difference. The results of these determinations are shown in Table I.

Purification of Globulin—The supernatant, representing the substances soluble in 0.14 M sodium chloride, was concentrated by being placed in a dialysis bag before a fan in a tight box containing Drierite. It was concentrated to about one-sixth of its original volume. The concentrated crude extract was dialyzed against distilled water until salt-free.

### Table I

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight of sample</th>
<th>Weight of residue</th>
<th>Per cent extracted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus 1</td>
<td>1.961 gm.</td>
<td>1.450 gm.</td>
<td>26.1</td>
</tr>
<tr>
<td>&quot; 2</td>
<td>8.687 gm.</td>
<td>6.300 gm.</td>
<td>27.4</td>
</tr>
<tr>
<td>&quot; 3</td>
<td>8.098 gm.</td>
<td>5.970 gm.</td>
<td>26.3</td>
</tr>
<tr>
<td>Liver 1</td>
<td>2.478 gm.</td>
<td>1.509 gm.</td>
<td>39.1</td>
</tr>
<tr>
<td>&quot; 2</td>
<td>3.376 gm.</td>
<td>1.696 gm.</td>
<td>44.1</td>
</tr>
<tr>
<td>&quot; 3</td>
<td>10.858 gm.</td>
<td>6.220 gm.</td>
<td>42.7</td>
</tr>
</tbody>
</table>
Some precipitation was observed, but greater precipitation occurred when the dialysis system was adjusted to pH 5.0 to 5.3 with a few drops of hydrochloric acid. The protein precipitated in this manner was taken into

![Descending Ascending]

**Fig. 1.** Electrophoretic pattern of crude 0.14 M sodium chloride extract of thymus nuclei; pH 8.55 at 17,700 seconds, potential gradient, 4.4 volts per cm.

![Descending Ascending]

**Fig. 2.** Electrophoretic pattern of thymus globulin; pH 3.33 at 5400 seconds, potential gradient, 3.0 volts per cm.

![Graph](http://www.jbc.org/)  

**Fig. 3.** Thymus and liver globulins; mobility plotted as a function of pH

solution in 50 to 60 ml. of 0.14 M sodium chloride solution adjusted to pH 8.5 with a Veronal buffer, centrifuged, and dialyzed against distilled water adjusted to pH 5.0 to 5.3. The protein was purified by repeating this procedure several times. Negative Molisch tests and absorption data indicated the absence of carbohydrates or nucleic acids in the purified globulin. The major portion of protein present in the crude extract was precipitable by half saturation with ammonium sulfate.
**Electrophoresis of Protein Solutions**—The 0.14 M sodium chloride extracts of both liver and thymus nuclei were analyzed in a Klett electrophoresis apparatus with buffer systems at an ionic strength of 0.1 as suggested by Miller and Golder (9). Fig. 1 shows the patterns of the thymus extract. The extract from liver gave essentially the same pattern, showing the presence of one major component. (This component was greatly diminished after precipitation of the globulin by dialysis against distilled water adjusted to pH 5.0 to 5.3.) The purified globulin migrated as a single component at all pH values. Fig. 2 shows a pattern of the globulin of thymus nuclei. Fig. 3 shows the mobilities of the globulins from thymus and liver nuclei at various pH values.

**DISCUSSION**

In the study of constituents of cell nuclei prepared by the Behrens procedure, the criteria of purity of the nuclear preparations are paramount. It was found that, after grinding dried tissue in a ball mill for a short time, nuclei with adhering cytoplasmic tabs were obtained. The cytoplasm was detectable by smear staining with the stains described. After longer periods of grinding, the distinguishable cytoplasmic material was very slight. The fact that about 26.5 per cent of the thymus nucleus and about 42 per cent of the liver nucleus are extractable with 0.14 M sodium chloride is evidence in itself that the material does not all come from extranuclear contamination, but must indeed come from within the nucleus.

Nuclei were extracted with 0.14 M sodium chloride to avoid the viscous solutions resulting from solution of the nucleohistone. The nucleohistone is soluble at higher or lower salt concentrations. The pH of the extraction mixture ranged from 6.0 to 6.5. Lipoprotein which is soluble at higher pH values was therefore not taken into solution. The nucleohistone and lipoprotein can be prepared from the residue of the 0.14 M sodium chloride extraction.

The 0.14 M sodium chloride extract shows a predominance of one electrophoretic component. It was found to represent a major part of the non-dialyzable material of the crude extract of thymus and liver nuclei. The insolubility of the major portion of the material of the crude extract in half saturated ammonium sulfate solution and the insolubility of the main component of the crude extract near its isoelectric point strongly suggests that it is a euglobulin.

The purified globulins of thymus and liver nuclei each moved as a single component during electrophoresis. The fact that the two mobility curves do not coincide suggests that these two proteins (from thymus and liver) differ somewhat. Two-dimensional paper chromatograms of the hydrolysates indicated that the two globulins are similar in amino acid composition.
SUMMARY

An appreciable quantity of proteins was extracted with 0.14 M sodium chloride solution from calf thymus and calf liver nuclei. A major component of the extractable proteins was isolated and shown to have the characteristics of a globulin.

BIBLIOGRAPHY

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