A CHEMICAL METHOD FOR THE ISOLATION OF
HELa CELL NUCLEI AND THE NUCLEAR LOCALIZATION
OF HELa CELL ALKALINE PHOSPHATASE

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Several chemical methods for the isolation of cell
nuclei have recently been described. Calf thymus
nuclei have been isolated by mild hypotonic
shock followed by Ficoll gradient purification
(1). A nonaqueous technique has been employed
by Hammel and Bessman for purifying duck
eythrocyte nuclei (2). Cell nuclei from cat brain
have been obtained by using a detergent, Triton
X-100, which results in a 2.8-fold enrichment of
nuclei (as measured by the increase in the ratio
of DNA/protein) (3). Triton X-100 has also been
used to isolate rat liver nuclei, and in this prepa-
ratment about 5-fold enrichment was obtained (4).
Citric acid at pH 3.5 has been used to prepare
HELa cell nuclei, which were reported to appear
uncontaminated by cytoplasmic material under
the phase microscope (5).

In the present report the isolation of HELa
cell nuclei with sodium deoxycholate is described.
The purity of the nuclei was evaluated by com-
paring the DNA-to-protein ratios of the whole
cells to those of the separated cell fractions (6).
The activity of β-glucuronidase was used as a
measure of the lysosomal contamination of the
various cell fractions. Assays of these cell fractions
revealed that most of the alkaline phosphatase
activity was associated with the nuclear fraction.

MATERIALS AND METHODS

Cell Cultures and Media

The methods and media used in this laboratory for
monolayer and suspension cultures have been de-
scribed (7, 8). Two different HELa cell lines were
studied: HELa Ch, a cell line having a high constitu-
tive level of alkaline phosphatase, and HELa Sb, a line
with low alkaline phosphatase activity which can be
increased from 3- to 20-fold by growth in medium
containing prednisolone (Δ1-hydrocortisone) (7). In
each experiment carried out with HELa Sb cells,
replicate cultures were grown in medium with and
without 1.0 µg/ml prednisolone phosphate. Mono-
layer cultures were harvested after 70 hr growth. The
cells were removed from the glass surfaces by brief
treatment with a solution of 0.025% trypsin (Micro-
biological Associates, Inc., Bethesda, Md., 1:200
Trypsin solution) and 3 × 10⁻⁴ M ethylenediamine
tetraacetic acid (EDTA) as previously described (7).
The action of trypsin and EDTA was stopped by sus-
pension in growth medium.

Nuclear Isolation Technique

All procedures were carried out at 0°C. The cell
suspension obtained from a Roux bottle (8.0 ± 0.2 ×
10⁶ cells) was centrifuged at 900 g (International
Refrigerated Centrifuge, Yoke No. 253, International
Equipment Co., Needham Heights, Mass.) for 10 min, and the pellets were washed once with cold saline. The cell pellet was then resuspended in 15 ml of ice-cold 0.8% saline containing \(10^{-3}\) M Tris hydrochloride, pH 7.4 (ST buffer). A 2.5 ml aliquot of whole cells was removed for assay of protein, RNA, DNA, and the activities of several enzymes. An ice-cold solution of 0.2% sodium deoxycholate in ST buffer (12.5 ml) was added slowly to the remaining 12.5 ml of cell suspension with gentle agitation with a glass rod. The resulting suspension was stirred 3 or 4 times during an 8 min period and then centrifuged at 900 g for 9 min. The supernatant or "cytoplasmic solution" was decanted and the "nuclear" pellet taken up in 10 ml of ST buffer (nuclei = \(6.6 \pm 0.3 \times 10^7\)). The difference between the total number of cells used and the number of nuclei recovered probably reflects nuclear clumping, which interferes with accurate nuclear counts although a small amount of nuclear lysis cannot be excluded. Examination of the nuclear preparation by light and phase microscopy shows the nuclei to be free of observable cytoplasmic tabs. Whole cells and nuclei were also fixed in 90% methanol–10% formalin and dried with gentle heating on glass slides which were previously dipped in water. Cells and nuclei were stained either with Giemsa's or histochemically for alkaline phosphatase as previously described (7).

**Assay Procedures**

Since it has been found that high concentrations of deoxycholate interfere with the diphenylamine reaction for DNA (9), the whole cell aliquot and the nuclear suspension were divided into two portions. The first portion was treated with an equal volume of 1% sodium deoxycholate and used for enzyme assays (10). The second portion was treated with an equal volume of 30% trichloroacetic acid and the precipitate sedimented at 1500 g. The precipitate was digested in 0.5 n perchloric acid at 75°C for 30 min, and the solution was clarified by centrifugation and assayed for DNA (9) and RNA (11). Calf thymus DNA and yeast RNA were used as standards. Alkaline phosphatase, acid phosphatase, and β-glucuronidase were assayed spectrophotometrically as previously described (10).

**RESULTS**

Fractionation of HeLa Ch cells was performed in 10 separate experiments. Table I summarizes the data from one experiment. It is apparent that alkaline phosphatase activity is almost totally confined to the nuclear fraction and that the increase in its specific activity (alkaline phosphatase activity/milligram of protein) parallels the enrichment factor for DNA (DNA content/milligram of protein). In all experiments, purification of alkaline phosphatase and enrichment of DNA were greater than 3.0-fold. The average purification for alkaline phosphatase in the 10 experiments was about 5-fold, and the enrichment of DNA about 4-fold. β-Glucuronidase assays performed on all cell fractions indicated that the nuclear fractions had less than 5 to 10% of the total cell enzyme activity. The low RNA content of the isolated nuclei is presumably a reflection of the purity of the nuclear fraction. A small amount of the total cell acid phosphatase activity was consistently found in the nuclear fraction. However, about 85% of the total cell acid phosphatase activity appeared in the cytoplasmic fraction.

<table>
<thead>
<tr>
<th>Table I Distribution of Phosphatases, DNA, and RNA in Subcellular Fractions Prepared from HeLa Ch Cells</th>
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<td><strong>Fraction</strong></td>
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<td>Nuclei</td>
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*μmoles of p-nitrophenyl phosphate hydrolyzed/15 min.  
†μmoles of p-nitrophenyl phosphate hydrolyzed/mg of protein.  
‡Fold increase of alkaline phosphatase/mg of protein.  
§Fold increase of DNA/mg of protein.  
¶Deoxycholate interference with assay.
Table II summarizes findings from one of four experiments on the isolation of nuclei from HeLa S3 cells grown in the presence (induced) and absence (control) of prednisolone. The results obtained on HeLa S3 cells grown in suspension cultures were similar to those obtained on cells in monolayer cultures. For both uninduced and induced HeLa S3 cells, the enrichment of DNA closely parallels the purification of alkaline phosphatase in the nuclear fraction (Table II). In uninduced HeLa S3 cells, the amount of enrichment of DNA and the purification of alkaline phosphatase were similar to those seen in HeLa Ch cells. Induced HeLa S3 cells, however, showed less enrichment of both DNA and alkaline phosphatase. This finding is probably a result of a hormone-induced alteration in the cell membrane which renders it more resistant to deoxycholate lysis (12). The biochemical data agreed with cytological observations. The nuclear preparations appeared to consist of at least 95% nuclei by light and phase microscopy. None of the nuclei had observable adhering cytoplasm. Histochemical studies have shown that alkaline phosphatase activity is present in the nuclei of most animal and plant cells (7, 15). The presence of alkaline phosphatase in the nuclei of animal cells in culture and its concentration on the chromosomes during cell division have been challenged as diffusion artifacts which arise during the staining procedure (15). However, the results obtained in this study which indicate that most of the alkaline phosphatase is found in the nuclear fraction of these HeLa cells support the histochemical evidence for nuclear localization of this enzyme.

**Discussion**

The results indicate that the nuclear fraction obtained from HeLa cells by a simple chemical method employing 0.1% sodium deoxycholate is relatively free of cytoplasmic material. The lipophilic properties of this anionic cholic acid derivative solubilize most of the cytoplasm and leave the nucleus intact. The primary advantages of this method are its simplicity and reproducibility. Deoxycholate has been used previously for separating microsomal membranes and ribosomes from the microsomal fraction of rat liver (13) and rat kidney cells (8). Recently, mechanically prepared HeLa nuclei were freed of adhering cytoplasmic contaminants by brief treatment in a solution of 0.5% sodium deoxycholate and 1.0% of the nonionic detergent, Tween 40 (14).

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The physiological basis for nuclear localization of alkaline phosphatase is not known. However, since mammalian alkaline phosphatase has been shown to have inorganic pyrophosphatase activity (16), it is possible that this enzyme could drive the reversible DNA and RNA synthesizing process.
reactions to completion by cleaving inorganic pyrophosphate which is one of the products of these reactions.

SUMMARY

A simple, rapid chemical technique for isolating HeLa cell nuclei is described. The nuclei are relatively pure as judged by biochemical and histochemical observations. Alkaline phosphatase is located predominately in the nucleus of HeLa cells.

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REFERENCES


