A RAPID METHOD FOR THE ISOLATION AND COLLECTION OF NUCLEI FROM WHOLE CELL SUSPENSIONS

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INTRODUCTION

The cytolytic action of certain surface-active agents led to the observation that some of the non-ionogenic ethanoxy compounds lyse the plasma membrane of amphibian erythrocytes and liver parenchyma cells more rapidly than the nuclear membrane (1). Plasmolysis by these substances might provide the basis of a technique for the isolation and collection of nuclei from cell suspensions that may prove to be more rapid and less damaging than the usual methods which involve the mechanical disruption of the cell. Furthermore, isolation of nuclei by a cytolytic method is easily controlled, may be followed microscopically, and can be undertaken when the number of cells involved is relatively small.

The surface-active agent used in this investigation was a non-ionogenic ethanoxy compound—an iso-octyl phenol condensed with an average of six ethylene oxide groups—known as Nonidet P40 (NP40) and provided by the Shell Co. of Australia, Shell House, Sydney, N. S. W. One advantage of this and allied compounds is their solubility in both aqueous and some non-aqueous media. The plasma membrane is lysed in both types of media when NP40 is present, and the rate of lysis can be increased so that the membrane breaks down and the nuclei are liberated while the cells are being centrifuged through a layer of medium containing NP40 into layers of higher density which do not contain the cytolysing agent. The isolated nuclei may be collected as a pellet or as a layer at a particular density of the suspension medium.

METHODS

Nucleated erythrocytes from the Queensland cane toad (*Bufo floridensis*) were washed several times and resuspended in ice-cold amphibian Ringer-phosphate solution at pH 7.0. Four methods of isolation were employed.

1. Isolation in a saline medium. Erythrocytes suspended in Ringer's solution were lysed by the addition of NP40 (final conc. 0.1 per cent). The suspension of free nuclei was centrifuged at 4000 *g* for 20 minutes. The nuclear pellet was freed from contamination by washing in several changes of medium in which the nuclei were resuspended by gentle mechanical agitation.

2. Isolation in a sucrose medium. Cells suspended in 0.25 M sucrose were lysed by the addition of NP40, and the nuclei were collected as a pellet after centrifugation. The nuclei were resuspended in 0.25 M sucrose and the procedure repeated until cytoplasmic debris was removed.

A more rapid and more convenient method was to centrifuge the cells in a discontinuous sucrose gradient, the uppermost layer of which contained 0.1 per cent NP40 (Fig. 1 *A*).

Erythrocyte nuclei isolated in sucrose medium by the methods described above contain some haemoglobin. The haemoglobin appears to be a surface contaminant since it is not present in "non-aqueous" nuclei (method 4) and does not pass through a small "non-aqueous" zone if such a zone is interposed below the cytolytic layer in the sucrose gradient.

3. Isolation in an aqueous, and suspension in a non-aqueous, medium. Mixtures of cyclohexane and carbon tetrachloride were used to form a non-aqueous density gradient. A layer of 0.15 M sucrose-Ringer solution containing 0.1 per cent NP40 was pipetted onto the surface of the non-aqueous medium.

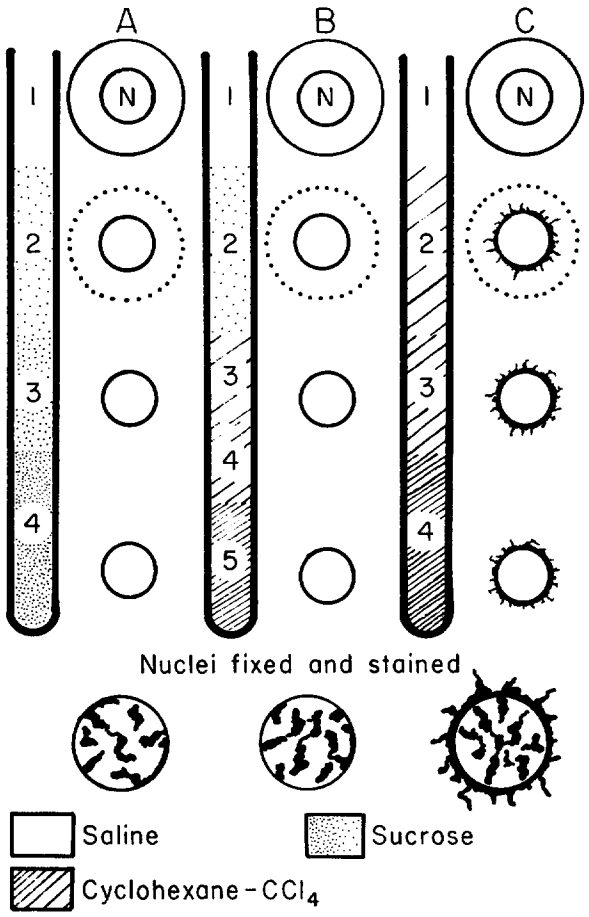


FIGURE 1 Schematic distribution of media in the centrifuge tube and diagrammatic representation of the erythrocyte and the isolated nucleus with reference to isolation methods 2, 3, and 4 (see text).

A. Isolation in a sucrose gradient. *1*, Erythrocytes suspended in amphibian Ringer-phosphate solution. *2*, Cytolysing layer, 1.5 M sucrose-saline containing 0.1 per cent NP40. *3*, 0.2 M sucrose. *4*, 0.25 M sucrose.

B. Isolation in sucrose-saline solution, suspension in cyclohexane-carbon tetrachloride mixtures. *1*, Erythrocytes suspended in amphibian Ringer-phosphate solution. *2*, Cytolysing layer, 1.5 M sucrose-saline containing 0.1 per cent NP40. *3*, *4*, *5*, Layers of cyclohexane-carbon tetrachloride mixtures of increasing density.

C. Isolation and suspension in cyclohexane-carbon tetrachloride mixtures. *1*, Erythrocytes suspended in amphibian Ringer-phosphate solution. *2*, Cytolysing layer, cyclohexane-carbon tetrachloride mixture (3:1.3) containing 0.5 per cent NP40. *3*, *4*, Layers of cyclohexane-carbon tetrachloride mixtures of increasing density.

FIGURE 2 Normal erythrocytes in amphibian Ringer-phosphate solution, pH 7.0. Phase contrast equipment. Dark field. × 700.

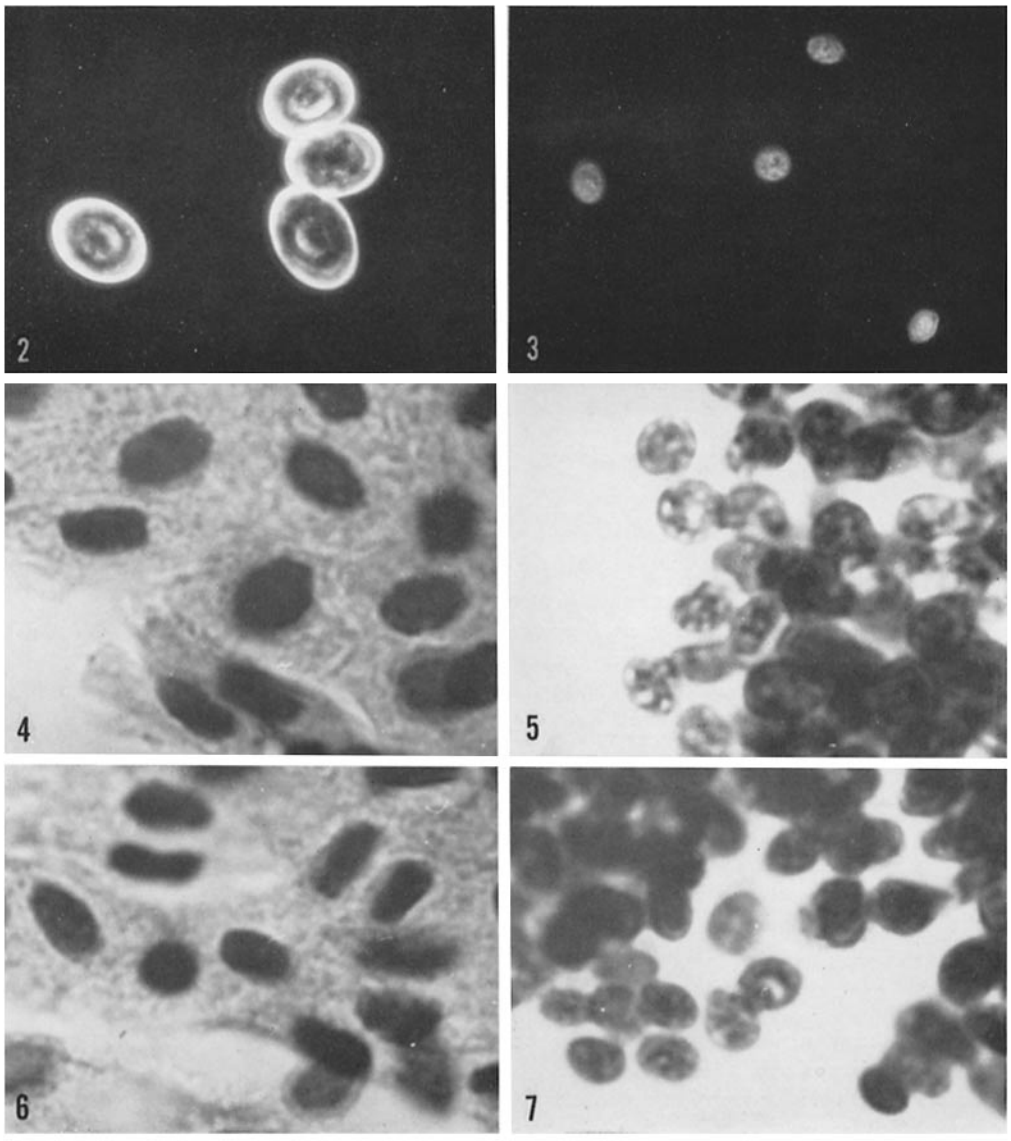
FIGURE 3 Erythrocyte nuclei in amphibian Ringer-phosphate solution containing 0.1 per cent NP40, pH 7.0. Phase contrast equipment. Dark field, as above. × 700.

FIGURE 4 Paraffin section, 5 μ, of erythrocyte pellet treated as described for Fig. 1 *A* (sucrose gradient). No cytolysing agent present. Weigert's haematoxylin and picro orange. × 1,000.

FIGURE 5 Paraffin section, 5 μ, of nuclear pellet, sucrose gradient containing cytolysing agent NP40, 0.1 per cent, as described for Fig. 1 *A*. × 1,000.

FIGURE 6 Paraffin in section, 5 μ, of erythrocyte pellet treated as described for Fig. 1 *B* (aqueous isolation and non-aqueous suspension). No cytolysing agent present. × 1,000.

FIGURE 7 Paraffin section, 5 μ, of nuclear pellet treated as described for Fig. 1 *B* (cytolysing agent, 0.1 per cent NP40, in aqueous layer). × 1,000.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Erythrocytes suspended in Ringer's solution were added to form the uppermost layer (Fig. 1*B*). During centrifugation, the nuclei are liberated in the sucrose-Ringer solution and pass from this into the non-aqueous layers. The nuclei may be collected as a pellet or as a layer at a particular density of the medium.

4. Isolation and suspension in a non-aqueous medium. In this case, the cytolysing zone was a cyclohexane-carbon tetrachloride mixture containing 0.5 per cent NP40. Erythrocytes in Ringer's solution were pipetted onto the surface of this zone and centrifuged as soon as possible so as to minimise the diffusion of NP40 into the aqueous layer (Fig. 1 *C*). Nuclear isolation took place during centrifugation, but subsequent examination of the nuclear pellet showed that a layer of cytoplasm consistently adhered to the nucleus. This contamination has not been eliminated as yet, although it appears to be decreased when the erythrocytes are first suspended in a calcium-free mixture of versene and Ringer's solution.

The nuclear pellets obtained by these procedures were fixed in formol-acetic-alcohol, washed, dehydrated, and embedded in paraffin. Sections were cut at 5μ and stained with Weigert's haematoxylin and picro orange G.

DISCUSSION

Nuclei isolated in a saline medium by this technique appear to be "clean" and morphologically normal under light microscopy. The erythrocytes (Fig. 2) are lysed almost instantaneously when NP40 is added to the suspension, while the nuclei (Fig. 3) remain apparently unchanged for some time.

"Sucrose" nuclei resemble the "saline" nuclei both under phase contrast microscopy and when the pellet is sectioned and stained. When a section of the nuclear pellet from a sucrose isolation (Fig. 5) is compared with a section of the control pellet obtained in the absence of NP40 (Fig. 4), the nuclei appear clean and the haematoxylin-positive material has essentially the same pattern and distribution in the fixed nucleus. The nuclear shrinkage which is noticeable in the fixed material is probably a fixation effect.

The advantages of isolation of nuclei through a density gradient are not so apparent with erythrocytes as with other cells in which cytoplasmic components may lend themselves to centrifugal stratification during the isolation process.

One of the advantages claimed for "non-aqueous" nuclei is that water-soluble components are retained, whereas a disadvantage is the loss of lipid-soluble substances. While no claim is made to present a method that has all the advantages and reduces the disadvantages of non-aqueous isolation, the following approach, in which these experiments are but the initial steps, may prove to be useful. If plasmolysis is induced in a sucrose medium above a non-aqueous phase (Fig. 1 *B*), then for a short time—the length of which can be modified by experimental design—the nucleus is in direct contact with an aqueous phase and the loss of water-soluble nuclear components would depend upon their rate of diffusion through the nuclear membrane and upon the rate at which the nucleus passed from the aqueous to the non-aqueous phase. Diffusion of nuclear components through the nuclear membrane is related to the molecular configuration and associations of the substance concerned, to the structure of the nuclear membrane, and to the concentration gradient. The latter may be experimentally controlled to a degree which will vary with the purpose of any particular isolation. In addition, the nucleus might be expected to retain an adsorbed aqueous layer on its surface, in much the same way that some insoluble particulate materials do when forced by centrifugation from an aqueous into a non-aqueous phase. Such a layer would modify the loss of lipid-soluble substances.

Stained sections of the control pellet (Fig. 6) and the nuclear pellet (Fig. 7) are essentially similar to sections obtained from sucrose isolation (Figs. 4 and 5).

When plasmolysis is induced in a non-aqueous phase containing NP40 (Fig. 1 *C*), the nuclei retain a thin and sometimes stranded covering of cytoplasm which is proving difficult to eliminate.

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REFERENCES

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