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disulphide bridges in carcinoembryonic antigen (Thomas et al., 1974). When carcinoembryonic antigen modified by treatment with 5.33 mm-metaperiodate was hydrolysed in oxygen-free conditions and subjected to acid hydrolysis cystine was detected in a subsequent amino acid analysis, whereas only cysteic acid was obtained from carcinoembryonic antigen treated with 0.533 mm-metaperiodate.

Oxidative cleavage of the disulphide bonds causes changes in the tertiary structure of the protein core of carcinoembryonic antigen and thereby disorients determinant groups, whether carbohydrate or protein, and hence decreases the ability of carcinoembryonic antigen to bind to the antiserum.

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A Rapid Procedure for the Isolation and Purification of Rat Liver Nuclear Envelope

JAMES R. HARRIS and JEREMY F. MILNE

Department of Physiology, University of St. Andrews, Bute Medical Buildings, St. Andrews, Fife KY16 9TS, U.K.

The principles previously developed for the production of nuclear envelopes from rat liver and hepatoma by Price et al. (1972) have been modified to enable this membrane material to be obtained rapidly from purified rat liver nuclei by using conventional centrifuge rotors rather than zonal rotors. The underlying factor which has been carried forward in the present method is that rat liver nuclei will swell and release their contents into solutions of low ionic strength in the absence of bivalent cations. A 1 mm solution of NaHCO₃ (pH 7.2) has been used to bring about this release of chromatin (Harris, 1974). In the method of Price et al. (1972) nuclear envelopes were isolated by rate zonal centrifugation direct from the 1000 g nuclear pellet after homogenization in 1 mm-NaHCO₃ buffer and overnight incubation at 4°C. When applied to purified nuclei, the 1 mm-NaHCO₃ treatment alone is not satisfactory for producing nuclear envelopes since an unmanageable gel forms as the chromatin is released from the bursting nuclei. This gel can, however, be dissociated by a brief treatment with DNAase (deoxyribonuclease I). The method given below outlines the details of a procedure which incorporates washing in 1 mm-NaHCO₃ together with a DNAase I treatment, and leads to the production of intact nuclear 'ghosts' and large torn sheets of envelope from purified rat liver nuclei.

All procedures were carried out at 4°C, unless stated to be otherwise. Purified nuclei were obtained from the livers of six Wistar rats after homogenization in 0.25 mm-sucrose-2 mm-MgCl₂-10 mm-Tris-HCl (pH 7.4) by using an Ultra-turrax type TP 18/2, and sedimentation of the 1000 g nuclear pellet through 2.2 mm-sucrose-10 mm-Tris-HCl (pH 7.4) at 100000 g for 2 h in a Beckman S.W. 27.1 rotor in an L2 65 B ultracentrifuge. The
pellets of nuclei were then washed twice at 1000g for 5min in the homogenization buffer. The combined pellets of purified nuclei were taken as the starting material for the isolation of nuclear envelope.

The nuclei were resuspended in 40ml of 1mm-NaHCO₃ (pH7.2) by shaking, and allowed to equilibrate for 5min before centrifuging at 31300g for 5min in a Beckman JA-20 fixed-angle rotor in a J-21 centrifuge. The pellet of slightly swollen nuclei was again resuspended in 1mm-NaHCO₃ by gentle syringing through a large-diameter needle, and re-centrifuged as above after a further equilibration period of 5min. A very swollen gelatinous pellet was obtained and this was dispersed by the addition of 1mm-NaHCO₃ containing DNAase I (Sigma Chemical Co., London S.W.5, U.K., type DN-100) to give 40ml of suspension with an enzyme concentration of 10µg/ml. This suspension was then incubated at room temperature (20°C) for 15–20min. During the incubation period the chromatin escaping from the burst nuclei is degraded by the DNAase, thus allowing the nuclear envelopes to become freely dispersed. The dissolution of the gelatinous state is extremely rapid and nuclear envelopes can be observed by phase-contrast microscopy to become freely suspended. The nuclear envelopes were then repeatedly washed in 40ml of 1mm-NaHCO₃ by centrifugation at 31300g for 5min. The release of chromatin throughout this sequence is shown in Fig. 1.

The nuclear envelopes from the final centrifugal wash were resuspended in 2ml of 1mm-NaHCO₃ and centrifuged at 500g for 5min. The small discoloured pellet was discarded and the white supernatant layered over a discontinuous sucrose gradient made up of 10ml of 2.0M-sucrose, 10ml of 1.8M-sucrose, 10ml of 1.5M-sucrose and 6ml of 0.25M-sucrose (all solutions made up in 10mM-Tris-HCl, pH7.4). The gradient was then centrifuged for 90min in a Beckman S.W. 27.1 rotor at 100000g (27000rev./min) in an L2 65B ultracentrifuge. A major band of purified nuclear envelope formed at the 1.5M/1.8M-sucrose interface during the centrifugation, as shown in Fig. 2. Prolonged centrifugation did not alter the position of this membrane band and isopycnic centrifugation for 20h at 100000g on a continuous sucrose density gradient likewise gave a single membrane band of flotation density 1.21±0.01. Gradient profiles have been recorded as a routine by direct photography and also by passing the contents of the tubes through an LKB Uvicord I1 u.v. absorptiometer coupled to a Bryans model 27000 chart recorder.

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Fig. 1 Release of chromatin from rat liver nuclei throughout 1mm-NaHCO₃ washes and DNAase treatment

Results were calculated from the absorbance values at 260nm of the 31300g supernatants.
Nuclear envelope was recovered from the absorptiometer effluent as 40-drop fractions or alternatively the membrane band was removed by hand from the gradients with a Pasteur pipette. Phase-contrast examination of this final membrane material revealed a free suspension of intact nuclear 'ghosts' and large sheets of torn envelope. The assessment was confirmed by electron microscopy by using thin sectioning and negative-staining after the removal of sucrose by washing twice with 1mM-NaHCO₃ at 31300g for 5min. The absence of mitochondria, rough and smooth membrane vesicles is readily apparent, only sheets of membrane and intact nuclear 'ghosts' showing the characteristic nuclear pore complexes, which can be regarded as morphological markers of nuclear envelope, are present.


**Ultrastructural Cytochemical and Metabolic Changes After Nodular Hyperplasia in Experimental Hepatomegaly**

NORMAN ROBINSON, JOHN G. NIEVEL and JOHN ANDERSON

Department of Anatomy, The London Hospital Medical School and Department of Medicine, King's College Hospital Medical School, London SE5 8RX, U.K.

Compounds of widely differing chemical structures induce drug-metabolizing enzymes and various morphological and biochemical changes which, after chronic administration, may lead to liver enlargement (Kunz et al., 1966). Phenobarbitone also induces a reversible liver growth and chronic treatment with high dose amounts produces hyperplastic