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Synthesis of Diphosphopyridine Nucleotide by Cell Nuclei isolated in Aqueous Media

It has recently been demonstrated that reasonably homogeneous preparations of cell nuclei can be isolated in good yield from rat or mouse liver[1]. By means of centrifugal fractionation of liver homogenates prepared in an aqueous medium (0·25 *M* sucrose — 0·0018 *M* calcium chloride), it has been possible to obtain 70–90 per cent of the nuclei with a minimum of cytological alteration in preparations containing by direct count less than 1 per cent of the intact cells of the tissue and less than 0·5 per cent of the free mitochondria of the homogenates.

The value of this method in cytochemical studies obviously depends on the permeability of the nuclear membrane. In this respect, Allfrey *et al.*[2,3] have contended that cell nuclei isolated in aqueous media are ill-adapted for studies of enzyme localization, primarily because extraction of water-soluble proteins is said to occur. These investigators have described a tedious method for the isolation of nuclei in organic solvents, utilizing desiccated tissue as the starting material. We are of opinion that it is unnecessary to resort to non-aqueous media.

Evidence for the integrity of nuclei isolated in aqueous media (other than their relatively high protein content[1]) has arisen from a study of the distribution in mouse liver homogenates of the enzyme catalysing the synthesis of diphosphopyridine nucleotide from nicotinamide mononucleotide and adenosine triphosphate. In a number of experiments, 69–101 per cent of the diphosphopyridine nucleotide-synthesizing activity of the homogenates was recovered in the nuclear fraction. Approximately 3 per cent of the original activity was found in the mitochondrial fraction, and the remainder was present in a fraction containing submicroscopic particles and the soluble material of the cytoplasm. The specific activity of the isolated nuclei (2·0–2·4 micromoles of diphosphopyridine nucleotide formed per hour per milligram of total nitrogen at 38°) was greater than that of the homogenates by a factor of 5–6. The higher recoveries of enzyme in the nuclear fraction (an average of 92 per cent in four consecutive experiments) were obtained when a step known to cause nuclear damage was eliminated from the fractionation procedure, and it seems entirely possible that the small amount of activity found under these conditions in the cytoplasmic fractions may have been the result of nuclear damage during the initial homogenization of the liver. When the isolated nuclei were disrupted by exposure to sonic oscillations[4] for 10 min. at 2°, approximately 85 per cent of the activity remained unsedimented after centrifugation for 30 min. at 130,000 times gravity.

The synthesis of diphosphopyridine nucleotide by liver cell nuclei is of interest for several reasons. The enzyme involved is a water-soluble protein that has been purified to a considerable extent[5]. Thus, at least in this one instance, the claim of Allfrey *et al.* with respect to the extraction of proteins from nuclei by aqueous media is not supported. Furthermore, so far as we are aware, the present finding comprises the first example of the clear-cut localization of an enzyme in isolated nuclei. Finally, since a number of diphosphopyridine nucleotide-linked dehydrogenases have been recovered largely in the cytoplasmic fractions[6], the synthesis of the coenzyme by isolated nuclei represents an example of an apparent biochemical interaction between nucleus and cytoplasm.

A more detailed description of the present work will be published elsewhere.

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Behaviour of Fertilized Rabbit Eggs exposed to Glycerol and to Low Temperatures

Mammalian spermatozoa, red blood-cells and ovarian tissue suspended in glycerol-containing media have been cooled to, and maintained for long periods at — 79° C. or — 190° C., and have resumed functional activity when thawed and inseminated, transfused or grafted[1–4]. The present study of the reaction of fertilized rabbit eggs to glycerol and to low temperatures was undertaken to extend these researches to a different and more active type of cell, and one which has not hitherto been found to survive exposure to low temperatures[5,6].

Fertilized eggs were recovered from the Fallopian tubes of rabbits 18–25 hr. after injection of chorionic gonadotrophin and artificial insemination. In preliminary experiments they were transferred to homologous serum containing 15 per cent glycerol at + 20° C. Under these conditions the eggs shrank down within the zona pellucida, presumably because of the hypertonicity of the medium. At + 5° C. the shrinkage was greater. When transferred successively to 2·5, 3·75, 5 and 7·5 per cent glycerol in serum at + 20° C., they contracted and re-expanded during one hour at each stage, suggesting that glycerol was penetrating into the eggs. In the course of treatment with 10 and 15 per cent glycerol, however, they shrank irreversibly, and when incubated in 15 per cent glycerol for 18 hr. or cultivated in serum after removal of glycerol by gradual stages or by washing, they degenerated. By contrast, fertilized rabbit eggs treated in successive 10-min. stages with 2·5, 3·75, 5, 7·5, 10 and 15 per cent glycerol in serum at + 37° C. contracted only slightly and quickly re-expanded, suggesting that the ova were more permeable to glycerol at + 37° C. than at + 20° C. The glycerol was then removed at + 37° C. by