*Bull. Inst. Zool., Academia Sinica* 10(2): 73-75 (1971)

# THE ISOLATION AND PURIFICATION OF NUCLEI FROM HOUSE FLY PUPAE

K. Y. JAN AND RUEY-MING LOOR

Institute of Zoology, Academia Sinica, Nankang, Taipei, Taiwan, Republic of China

*Received for publication, October 1971*

## ABSTRACT

**K. Y. Jan and Ruey-Ming Loor** (1971) *The Isolation and Purification of Nuclei from House Fly Pupae*. Bull. Inst. Zool. Academia Sinica 10(2): 73-75. Nuclei of house fly pupae were isolated in aqueous medium by homogenization, filtration, washing and density gradient centrifugation. They are intact visually, but some five percent of cytoplasmic contamination still exists in the final nuclear preparation. The isolated nuclei are of circular shape, range from $7.0\times7.0$ to $30\times30\ \mu^2$ in size.

Most if not all, the genetic materials are included in the nucleus. Nucleus as it is composed of DNA complex with protein and RNA is of interest inasmuch as in this condition that the nucleus carries out it's DNA replication and RNA synthesis and that the cell multiplies and genetic information expresses. However, it is generally accepted that only a small portion of the neclear DNA of each type of cell are transcribed at a time. The biologists always want to know who makes the decision? what portion of the nuclear DNA and when to be transcribed? and how? To approach these problems one would first think of simplifying the problems by single out the nucleus from the cellular complex. The isolation of nuclei as such should then be important in studies on histones and other nuclear proteins, nuclear enzymes, nuclear DNA RNA and nucleolar components.

The house fly is used as experimental animal, because it is easy to rear, of short life cycle, and with many genetical and cytological advantages (7,8). In particular, the house fly is one of the insects having four distinct metamorphic stages. This would provide a unique system for studying gene regulation in the developmental processes.

In this communication we report the technique for isolation and purification of nuclei in large quantities from the house fly pupae.

## MATERIALS AND METHODS

The adults of *Musca domestica* L. were kept at $22\pm1$°C and fed with adult medium (6 gm skim milk powder and 6 gm dry yeast in 60 ml of distilled water) soaked in cotton. Eggs were inoculated in the larval medium (40 gm skim milk powder, 40 gm dry yeast and 7 gm agar cooked with 400 ml tap water) and kept at $30\pm1$°C. Under this cultural condition, the eggs hatched in 8-12 hours after deposition, larval stage lasted about 5 days, pupae maintained at $22\pm1$°C took about

7 days to emerge, the adults mated by the third day after emergence and the first bunch of egg deposited two days after mating. Pupae about 3 days old were used for the present experiments.

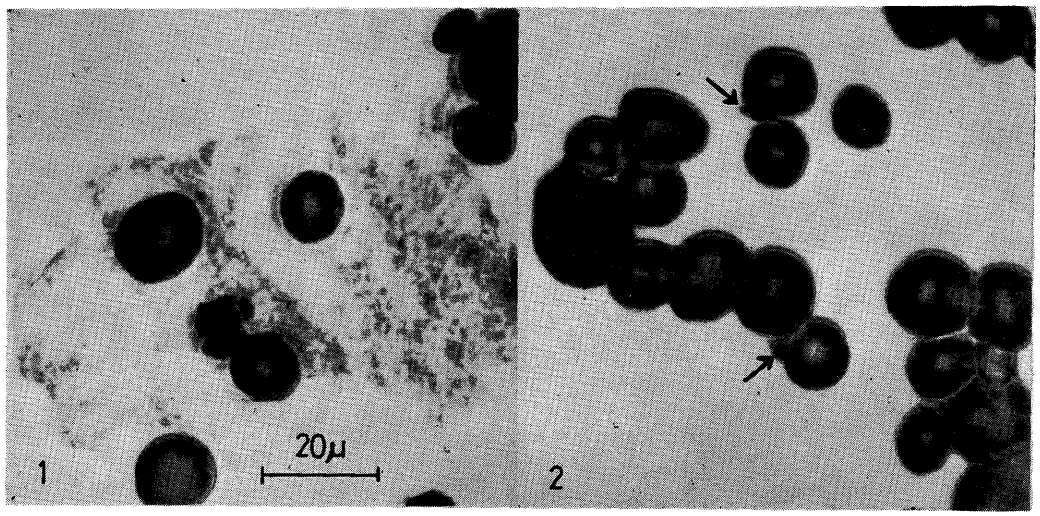
The isolation and purification of nuclei were carried out at 4°C and the whole procedure took 6 hours. Pupae 30 gm in 150 ml of nuclear medium (2% sodium citrate, 0.1M sucrose, 1mM calcium chloride, 1mM magnesium chloride, 0.05% Triton X-100 and 0.1M disodium ethylenediaminetetraacetate dihydrate, pH 5.8) were homogenized at low speed (20th division) for 5 minutes in a VirTis 45 homogenizer. The homogenate was filtered through a layer of nylon cloth (pore size $85\times76\mu^2$). The filtrate was centrifuged at 500 rpm for 5 minutes in a Marusan medium rotor. The pellets after six washes were again suspended in the nuclear medium and layered over the centrifugal medium (1.8M sucrose and 3mM calcium chloride) and spun at 10,000 rpm for 5 minutes in a Hitachi Ultracentrifuge 65P with RPS25-2A rotor. Sample from each step was taken, stained with 0.5% safranin O and examined under a Leitz phase contrast microscope.

## RESULTS AND DISCUSSION

The relative ease or difficulty that one encounters in isolating nuclei is a function of several factors, the most crucial of which is that the degree of difficulty of liberating nuclei from cytoplasm. Figure 1 shows that some of the nuclei were already singled out after six washes in nuclear medium. The homogenization condition used in the present experiments sufficiently ruptured the cells, yet left the nuclei undamaged. Squeezing the homogenate through a layer of nylon cloth was found to be better than through a few layers of cheesecloth, as such the fiber contamination was eliminated and the loss of nuclei was reduced.

A theoretically perfect nuclei isolation should yield a product which contains no cytoplasmic contamination and has not lost any nuclear component during the isolation. The nonaqueous solvent techniques unavoidably damage the nuclear membrane, whereas the aqueous methods cause a leakage of soluble nuclear components (10). The use of detergents causes a sufficiently attack on the cytoplasmic matrix so that it can be removed from nuclei by homogenization, but detergents damage the nuclear membrane and favor the release of enzymes which tend to destroy nuclei (1). For instance, the Tritons appear to disrupt the mitochondria and lysosomes during homogenization, a process which releases enzymes attacking macromolecules of chromatin and thus renders nuclei nongelable (9). The introduction of some agents which can suppress these enzymes in order to stablize the nuclei and prevent loss of nuclear macromolecules is then highly desirable. For this purpose the divalent cation $Ca^{++}$ and $Mg^{++}$ have been added into the nuclear medium. Wang (10) claimed that calcium reduces the fragility of nuclei probably by hardening the nuclear membrane and prevents (minimizes) nuclear clumping or gel formation and calcium also serves to inactivate mitochondria by uncoupling oxidative phosphorylation. Magliozzi *et al.* (9) also reported that magnesium chloride throws the nuclei into a more condensed state, and reduces cytoplasmic agglutination. In the absence of other enzyme inhibitors, however, $Ca^{++}$ and $Mg^{++}$ cause a loss of the easily extractable histone fraction from rat liver nuclei (4). Dounce and Ickowicz (5) recently discovered that $Pb^{++}$, $Cd^{++}$ and $In^{++}$ aid in stablizing nuclei in cellular homogenate by inhibiting the action of proteases and other enzymes. In addition, disodium ethylenediaminetetraacetate dissociates cells and inhibits DNase activity (6), citric acid helps the removal of cytoplasm from nuclear membrane (3), and sucrose tends to reduce the stickness of the nuclei (2).

The nuclei isolated in the present procedure are more or less of circular shape under the weight of a cover glass. Their sizes range from $7.0\times7.0$ to $30\times30\mu^2$. Under a phase contrast microscope the nuclear membrane is doubly refractile (Fig. 2) but the nucleoli are not visible. Starting with 30 gm of pupae, about 3 gm of purified nuclei were obtained. This nuclear preparation contains no more than 5% cytoplasmic contamination as estimated visually. The biochemical analysis of the DNA, RNA, acidic and basic proteins enclosed in these nuclei is undergoing at the moment.




Photomicrography of nuclear preparations, Fig. 1. some nuclei were freed from cytoplasm after 6 washes in nuclear medium; Fig. 2. some cytoplasmic debris (arrows) still exist at the final preparation.

## REFERENCES

1. Anderson, K.M., F.H. Lee and K. Miyai (1970) Effects of androgen *in vivo* on some properties of isolated rat ventral prostate nuclei. *Exptl. Cell Res.* **61**: 371-378.
2. Boyd, J.B., H.D. Berendes and H. Boyd (1968) Mass preparation of nuclei from the larval salivary glands of *Drosophila hydei*. *J. Cell Biol.* **38**: 369-376.
3. Busch, H. (1967) Isolation and purification of nuclei. *Methods in Enzymology* **12A**: 421-448.
4. Dounce, A.L. and R. Ickowicz (1969) The effect of the method of isolation of cell nuclei on the ratios of nuclear protein to DNA. *Arch. Biochem. Biophys.* **131**: 359-368.
5. Dounce, A.L. and R. Ickowicz (1970) The use of low concentrations of heavy metals in the isolation of rat liver nuclei. *Arch. Biochem. Biophys.* **137**: 143-155.
6. Glaeser, R.M., J.E. Richmond and P.W. Todd (1968) Histotypic selforganization by trypsin-dissociated and EDTA-dissociated chick embryo cells. *Exptl. Cell Res.* **52**: 71-85.
7. Jan, K.Y. and J.W. Boyes (1970) Differential DNA replication in the XX and XY cells of *Musca domestica* L. *ocra* strain. *Can. J. Genet. Cytol.* **12**: 461-473.
8. Jan, K.Y. (1971) The ending pattern of DNA replication in larval brain cells of house fly. *Bull. Inst. Zool., Academia Sinica* **10(2)**: 77-82.
9. Magliozzi, J., D. Puro, C. Lin, R. Ortman and A.L. Dounce (1971) Ratios of nuclear proteins to DNA for rat and mouse tumors and possible effect of cytoplasmic fibrils in isolating nuclei. *Exptl. Cell Res.* **67**: 111-123.
10. Wang, T.Y. (1967) The isolation and purification of mammalian cell nuclei. *Methods in Enzymology* **12A**: 417-421.