THE ISOLATION OF ENZYMICALLY ACTIVE NUCLEI FROM THE RAT HEART AND UTERUS

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Recent results obtained in many laboratories (see 1) have focused attention on the role of the cell nucleus in the action of growth-promoting hormones. In order to extend our studies on the action of thyroid hormone, growth hormone, and testosterone on liver RNA polymerase (2, 3) to other tissues, we have modified our method for the isolation of rat liver nuclei (4). We report here a method for the isolation of enzymically active nuclei from the rat heart and uterus.

EXPERIMENTAL

Isolation of Heart Nuclei

All procedures were performed at 0–4°. Hearts from at least five male Sprague-Dawley rats (150 ± 20 g body weight) were pooled and weighed in 0.32 M sucrose, 3 mM $MgCl_2$ (homogenizing medium, see 4); the tissue was minced finely with curved scissors and passed, using a stainless steel tissue press, twice each through sieves with pore diameters of 1.27 and 0.95 mm. The fragmented tissue was then homogenized in 3 volumes of homogenizing medium for 2 min 30 sec using an Ultra Turrax TP 18/2 tissue disintegrator (Janke und Kunkel K.G., Staufen 1. BR., Germany) run at 60 v (mains, 240 v). The homogenate was filtered through two layers of nylon bolting cloth (110 mesh, John Staniar Ltd., Manchester, England) and the nylon bolting cloth rinsed with an equal volume of homogenizing medium. 25 ml of homogenate were diluted with 7 ml of water, 15 ml of homogenizing medium were layered underneath, and a crude nuclear fraction was isolated by centrifugation at 700 *g* for 10 min. The pellet was resuspended to a final volume of 13 ml in 2.4 M sucrose–1 mM $MgCl_2$ using a ground-glass homogenizer (4). The purified nuclear fraction was isolated by centrifugation for 45 min at 50,000 *g* in the Spinco No. 40 rotor and usually suspended in 1.0 ml of 0.25 M sucrose–1 mM $MgCl_2$.

Isolation of Nuclei from the Uterus

All procedures were performed at 0-4°. Uteri from at least ten female Sprague-Dawley or Holtzmann rats (100–200 g) were pooled and weighed in the homogenizing medium, minced finely, and passed twice each through sieves with pore diameters 1.27, 0.95, and 0.63 mm, essentially as described above for heart nuclei. The fragmented tissue was homogenized in 3 volumes of homogenizing medium for 1 min 20 sec using the Ultra Turrax disintegrator as described

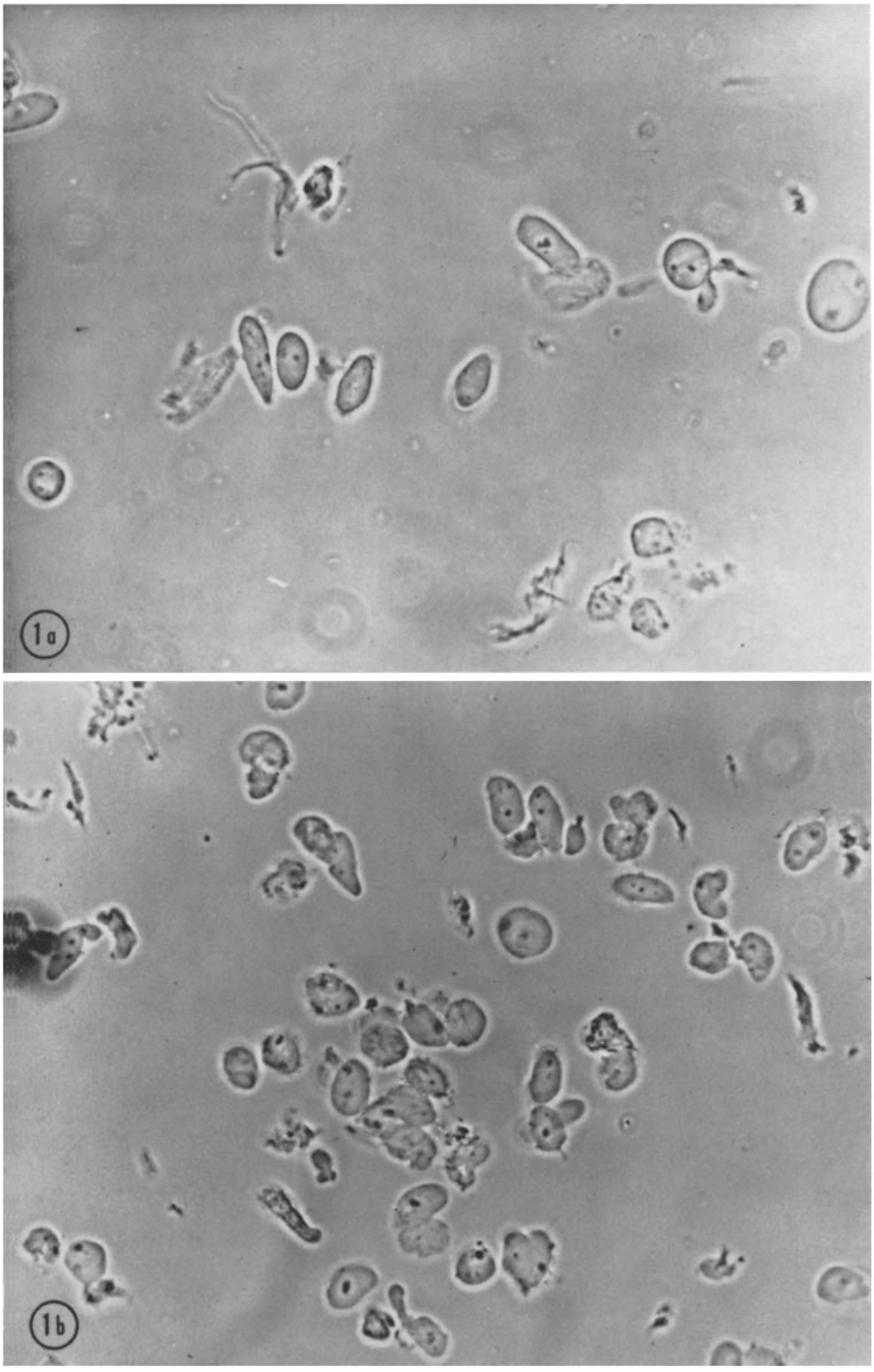

FIGURE 1 Phase contrast photomicrograph of nuclei isolated from (*a*) heart (× 750), and (*b*) uterus.

TABLE I

Protein and Nucleic Acid Contents of the Nuclear Preparations

	Protein: DNA	RNA: DNA	Recovery of DNA
			%
Heart homogenate	79.5	0.92	
Heart nuclei	4.3	0.23	43
Uterine homogenate	24.3	0.58	
Uterine nuclei	3.9	0.26	47
Liver nuclei (see ref. 4)	4.1	0.24	

above. The homogenate was filtered through nylon bolting cloth and the filter rinsed with an equal volume of homogenizing medium. The unfiltrable residue was removed from the bolting cloth with a spatula, and rehomogenized and filtered. 30 ml of the pooled filtrates were diluted with 8 ml of water, and 10 ml of homogenizing medium were layered underneath to yield a crude nuclear fraction after centrifugation at 700 *g* for 10 min. The pellet was resuspended to a final volume of 8 ml in 2.4 M sucrose–1 mM $MgCl_2$, and the purified nuclear fraction isolated by centrifugation for 45 min at 50,000 *g* in the 8 × 7 ml rotor of the MSE 40 preparative ultracentrifuge.

RESULTS

Morphological Appearance of the Nuclei

Because of the more severe methods necessary for homogenization of the tissue, the morphological appearance of the isolated nuclei was not so satisfactory as was observed for rat liver nuclei, using essentially the same procedure (4). Fig. 1 *a* shows a phase contrast micrograph of a typical preparation of heart nuclei, and Fig. 1 *b*, a typical preparation of uterine nuclei. Little cytoplasmic contamination is evident, although a few nuclei appear to be slightly damaged. Since our studies on the effect of various hormones on RNA synthesis by the nucleus (2, 3) required a high recovery of DNA, it was found necessary to compromise between damage to the nuclei and a low recovery of DNA. Under conditions in which only 10% of the DNA was recovered, the nuclei were found to have a morphological appearance indicating less damage and cytoplasmic contamination than seen in Fig. 1 *a-b*.

TABLE II

Specific Activities of the DNA-dependent RNA Polymerase and of NAD Pyrophosphorylase in the Nuclear Preparations

	Specific activity* of		
	RNA Polymerase		NAD pyrophosphorylase‖
	Mg^{2+}-activated reaction‡	Mn^{2+}-activated reaction§	
Heart nuclei	456	1355	99
Uterine nuclei	377	1235	69
Liver nuclei	926	3150	353

* Specific activities are expressed per mg DNA.
‡ μμmoles ^{3}H-ATP incorporated into RNA/15 min.
§ μμmoles ^{3}H-ATP incorporated into RNA/45 min.
‖ mμmoles NAD synthesized/20 min.

Chemical Composition

The DNA, RNA, and protein contents of the preparations, measured by methods described earlier (4), are shown in Table I. It is of interest to note that the protein:DNA and RNA:DNA ratios were essentially the same for nuclei isolated from the heart, uterus, and liver, even though the ratios for the homogenate showed a considerable variation (see also 4).

Nuclear Enzyme Activities

The DNA-dependent RNA polymerase in rat liver nuclei (5) exhibits differences in kinetics and the nature of the product formed when assayed in the presence of Mg^{2+} ions or $(NH_4)_2SO_4$ and Mn^{2+} ions (6, 7). The activity of RNA polymerase was, therefore, measured, under both reaction conditions, in nuclei from heart and uterus as for liver preparations (7) except that (*a*) the final concentration of [^{3}H]-ATP in the incubation medium was 2 mμmoles and that of nonradioactive ATP was 18 mμmoles, and (*b*) the medium contained 5 μmoles of phosphoenol pyruvate and 10 μg of pyruvate kinase. ^{3}H-labeled ATP (specific activity, 2.48 c/mmole) was purchased from Schwarz BioResearch, Inc., Orangeburg, N.Y. The methods for the determination of NAD pyrophosphorylase, cytochrome oxidase, and NADH–cytochrome *c* reductase were identical to those used for rat liver nuclei (4). The specific activities of RNA polymerase under both reaction conditions and of

NAD pyrophosphorylase, expressed per mg DNA, are shown in Table II; in all cases, the activity observed in heart and uterine nuclei was lower than that observed for liver nuclei. In addition, we have found that the activity of RNA polymerase in uterine nuclei varies during the oestrus cycle (8); the rats used in these experiments were selected at random, and, therefore, indicate an average value. The characteristics of the RNA polymerase reactions in isolated heart and uterine nuclei were essentially the same as observed for liver nuclei (7) and will be presented in detail elsewhere. The requirement of phosphoenol pyruvate and pyruvate kinase for the reaction may be explained by the ATPase activity of muscle fibers contaminating the preparations of isolated nuclei.

Cytoplasmic Enzyme Activities

The specific activities of cytochrome oxidase and NADH–cytochrome *c* reductase, expressed per mg DNA, in the preparation are shown in Table III. Less than 1% of the cytochrome oxidase activity of the homogenate and approximately 2% of the NADH–cytochrome *c* reductase activity was recovered in the preparations; although this activity of cytoplasmic marker enzymes was greater than observed for liver nuclei (4), the results are in general agreement with the results of morphological examination (see Fig. 1 *a-b*) which indicated a low level of cytoplasmic contamination.

TABLE III

Specific Activity of Cytoplasmic Enzyme Markers in the Nuclear Preparations

	Specific activity* of	
	Cytochrome oxidase‡	NADH–cytochrome *c* reductase§
Heart homogenate	39.300	4.67
Heart nuclei	0.288	0.067
Uterine homogenate	5.10	1.91
Uterine nuclei	0.031	0.043

* Specific activities are expressed per mg DNA.
‡ μmoles of cytochrome *c* oxidized/minute at the initial rate of reaction.
§ μmoles of cytochrome *c* reduced/minute at the initial rate of reaction.

DISCUSSION

The method described here for the isolation of enzymatically active nuclei from the rat heart and uterus is essentially the same as that described previously (4) for the isolation of rat liver nuclei. The procedure is also similar to a method described recently (9) for the isolation of skeletal muscle nuclei. Our method results in a preparation, which, though morphologically less pure, contains a higher percentage of the DNA in the homogenate; the advantages of a high recovery of DNA have been discussed elsewhere (4). The observation that the specific activities of the nuclear enzymes (Table II) were lower in preparations of heart and uterine nuclei than in liver nuclei, is generally consistent with results obtained in vivo (see 10), where it has been found that the protein synthetic activity of the heart and uterus is lower than that of the liver. It is unlikely that the lower enzyme activities were caused by the more drastic treatment used for homogenization, since it was found that the use of the Ultra Turrax for homogenization of liver had no effect on the specific activity of the nuclear enzymes.

Our isolation procedure may be adapted for the isolation of nuclei from smaller amounts of tissue, provided that a correspondingly smaller volume of 2.4 M sucrose medium is used for the final purification. In addition, it is necessary, when homogenizing with the Ultra Turrax, to ensure that the homogenate volume is not less than 5 ml. Under these conditions, it is possible to obtain essentially similar recoveries of DNA in the nuclear fraction, starting with as little as 500 mg of tissue. It is of interest to note in this context that we have consistently observed a higher recovery of DNA in uterine nuclei from ovariectomized rats, presumably because of the lower concentration of connective tissue in the ovariectomized rat uterus.

Ramuz et al. (11) have recently detected soluble RNA polymerase activity in nuclei isolated in 2.2 M sucrose alone, which they were unable to detect in nuclei isolated by a method analogous to that described here.[1] However, it is not yet possible to assess the physiological importance of this soluble RNA polymerase activity. Since the tightly bound RNA polymerase activity of nuclei isolated by the method described in this communication has been shown to reflect physiological changes in the rates of growth induced by various hormones

[1] P. Chambon, personal communication.

in the whole animal (2, 3, 12), it can be concluded that the method may be of further use in the investigation of the role of the nucleus in the control of cell growth.

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