COMPARISON AND CHARACTERIZATION OF NUCLEAR ISOLATION PROCEDURES AS APPLIED TO CHICK OVIDUCT

JOHN T. KNOWLER, HAROLD L. MOSES, and THOMAS C. SPELSBERG

From the Departments of Obstetrics and Gynecology and Pathology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232. The authors' present address is the Departments of Endocrine Research and Pathology, Mayo Clinic, Rochester, Minnesota 55901.

ABSTRACT

A number of methods for the preparation of chick oviduct nuclei have been compared. Nuclei have been isolated in hypertonic sucrose and citric acid and the product has been characterized with respect to cleanliness, ultrastructure, RNA polymerase activity, RNA integrity, and chromatin composition. The study demonstrates that the choice of oviduct nuclear isolation procedure will depend markedly on the purpose for which the nuclei are required. Thus, nuclei prepared entirely in high-molarity sucrose retain the highest levels of RNA polymerase. Those prepared rapidly in the presence of citric acid retain nuclear RNA in an essentially undegraded state. Finally, a bulk preparation is described which, because of its adaptability and high yield of morphologically intact nuclei using large amounts of tissue, is ideal for use in preparing chromatin. Conditions are described by which isolated nuclei can be stored for up to 6 months and retain their morphology, chemical characteristics, and RNA polymerase activity.

INTRODUCTION

Despite the multitude of methods reported for the isolation of nuclei, there would appear to have been no detailed characterization of the basic techniques with respect to their suitability for specific studies. During the course of investigations on the hormone-induced cytodifferentiation of the chick oviduct (for a review see O'Malley et al., 1969), we have become increasingly aware of this problem and describe here an analysis of oviduct nuclei made by a spectrum of reported techniques.

The isolation procedures described in this paper involve hypertonic sucrose and citric acid and were selected on a basis of popularity or because of current usage in our laboratories. The isolated nuclei were analyzed for chemical composition, species of RNA, endogenous RNA polymerases of both nucleolar and nucleoplasmic origin and examined for cleanliness and ultrastructure by electron microscopy. Further, the effects of the various procedures on chromatin composition, template capacity, as well as histone and acidic protein species are presented. It is our opinion that the analysis of these isolation methods by multiple techniques performed on the same tissue in the same laboratory allows more definitive conclusions concerning the usefulness of the various procedures.

MATERIALS AND METHODS

Source of Tissue

Oviducts were obtained from immature chicks (Rhode Island Reds) which had received daily injections (subcutaneous) of 5 mg diethylstilbestrol for 17 days. Such oviducts are fully developed and weigh about 1–2 g per chick. The oviducts were quickly trimmed of connective tissue and were either used immediately for isolation of nuclei or quick frozen in solid carbon dioxide and stored at −70°C until needed.

Isolation of Nuclei

All steps were performed at 0°–4°C unless otherwise specified. Purified nuclei were either used immediately or stored in 0.01 M Tris/HCl, pH 7.5, containing 25% (wt/vol) glycerol and 1 mM $MgCl_2$. The methods employed were: (I) 2 M sucrose method, (II) bulk method, (III) citric acid method, and (IV) $MgCl_2$/citric acid method.

(I) 2 M SUCROSE METHOD: The method is similar to that described by Chauveau et al. (1956) except that 2.2 M sucrose is replaced by 2.0 M sucrose to improve yield. Fresh or frozen oviducts were finely chopped and homogenized with 10 strokes of a Thomas Teflon pestle-glass homogenizer (Arthur H. Thomas Co., Philadelphia, Pa.) in 20 vol (milliliter per gram tissue) of 2.0 M sucrose in TKM buffer (0.05 M Tris/HCl, pH 7.5, containing 0.025 M KCl and 0.005 M $MgCl_2$). The homogenate was filtered through four layers of cheesecloth, layered over another 4 vol (milliliter per gram tissue) of the homogenization buffer, and centrifuged for 1 h at 70,000 *g*. The floating debris and the supernatant were discarded and the walls of the centrifuge tubes cleaned with tissue paper. The nuclear pellet was suspended by means of 10 strokes of a loose-fitting homogenizer in 2 vol (milliliter per gram tissue) of 1.7 M sucrose in TKM with or without 0.2% Triton X-100. The suspension was filtered through 100 mesh organza, layered over 2 vol (milliliter per gram tissue) of 1.8 M sucrose in TKM, and centrifuged for 20 min at 15,000 *g*. The time required for isolation was about 2 h and yields of nuclei were 40–60%.

(II) BULK PREPARATION: This method has been reported previously (Spelsberg et al., 1971) and is essentially a hypertonic sucrose method based on the rational of a number of similar methods (Widnell and Tata, 1964; Blobel and Potter, 1966). It is suitable for much larger amounts of tissue than most methods and can be used for the isolation of nuclei from many tissues including the liver, spleen, kidney, erythrocytes, brain, and lung of chickens and rats. The preparation requires about 1½ h to complete and yields are optimally 40–60% though they vary with the amount of starting material.

(III) CITRIC ACID METHOD: This was essentially the procedure described by Higashi et al. (1966). Up to 30 g of finely chopped oviduct was homogenized by 10–20 strokes of a motor-driven Thomas Teflon-glass homogenizer in 10 vol (milliliter per gram tissue) of 2.5% (wt/vol) citric acid. The homogenate was centrifuged at 600 *g* and the supernatant discarded. The nuclear pellet was resuspended in 10 vol (milliliter per gram tissue) of 0.25 M sucrose in 1.5% (wt/vol) citric acid and layered over twice the volume of 0.88 M sucrose in 1.5% (wt/vol) citric acid. After centrifugation for 10 min at 900 *g*, the supernatant was discarded and the pellet of nuclei retained for analysis. Final preparations were cleaner if this discontinuous gradient step was repeated. The pH values of the above homogenate and resuspended nuclear pellet were 2.5–2.6. The isolation required 1–½ h and the yield was 30%.

(IV) $MgCl_2$/CITRIC ACID METHOD: This method has been used successfully in the preparation of uterine nuclei with retention of high-molecular weight RNA (Knowler and Smellie, 1973) and has been slightly modified for the preparation of oviduct nuclei. 50 mg–5 g of finely chopped oviduct was homogenized in 1 mM $MgCl_2$ in a chilled Polytron PT-10 homogenizer (Brinkman Instruments Inc. Westbury, N.Y.) for 30 s at 36 V. The volume of added 1 mM $MgCl_2$ was kept as low as possible; 2 ml for amounts of tissue up to 800 mg and 2.5 ml/g for larger amounts. An equal volume of 0.1 M citric acid in 1 mM $MgCl_2$ was rapidly mixed with the homogenate which was filtered through four layers of cheesecloth. After centrifugation at 300 *g* for 5 min, the crude nuclear pellet was suspended in 2.5 vol (milliliter per gram tissue) of 0.05 M citric acid in 1 mM $MgCl_2$ containing 1.0% (wt/vol) Triton X-100 and mixed with an equal volume of 0.5 M sucrose in the same solution. The suspension was layered over 5 vol (milliliter per gram tissue) of 0.32 M sucrose in 0.05 M citric acid, 1 mM $MgCl_2$, 1.0% (wt/vol) Triton X-100 and centrifuged at 800 *g* for 5 min. The nuclear pellet was retained, the entire preparation having taken approximately 30 min with 40–50% yield. The pH of the above citric acid solutions was 2.7.

Analysis of Nuclear Constituents

RNA POLYMERASE: The assay of endogenous RNA polymerase activity was a modification of Roeder and Rutter (1970) and is described elsewhere (Glasser et al., 1972).

Analysis of the Species of RNA in Isolated Nuclei

In order to obtain sufficient specific activity in the nuclear RNA (nRNA) for polyacrylamide gel elec-

trophoresis, tissue slices were incubated with radioactive precursor in vitro.

Chicks were killed by cervical dislocation, the oviduct removed, divided in half longitudinally, and cut into small sections. Each chopped oviduct was incubated in a 25 ml conical flask in 4 ml of Eagle's medium containing 25 μCi/ml of [5-^{3}H]uridine. Incubations were for 1 h at 37°C under an atmosphere of 95% O_2/5% CO_2 in a shaking water bath. The tissue was then washed once in saline at 4°C and once with the first buffer of the appropriate nuclear preparation.

RNA was prepared by a modification of the method of Joel and Hagerman (1969). The extraction process was modified for isolation from chick oviduct nuclei in that the nuclei were lysed in 0.05 M sodium acetate (pH 5.2) containing 1% sodium dodecyl sulfate and 1 mg/ml bentonite before extraction with phenol. Contaminating DNA oligonucleotides remaining after deoxyribonuclease treatment were not exhaustively removed from the preparation since they were run off the end of the polyacrylamide gels. The separation of RNA on polyacrylamide gels has been previously described (Knowler and Smellie, 1971).

Preparation and Characterization of Chromatin

Chromatin was isolated and analyzed as previously described (Spelsberg and Hnilica, 1971: Spelsberg et al., 1971). Histone species were analyzed by electrophoresis on polyacrylamide gels as described by Panyim and Chalkley (1969), and acidic proteins were resolved on polyacrylamide gels by the methods of Wilson and Spelsberg (1973). The measurement of the template capacity (for DNA-dependent RNA synthesis) of the isolated chromatin using bacterial RNA polymerase (Burgess, 1969) has also been reported (Spelsberg and Hnilica, 1971; Spelsberg et al., 1971).

Chemical Analysis

DNA was assayed by the method of Burton (1956), RNA was measured by the orcinol reaction (Ceriotti, 1955), and protein was estimated by the method of Lowry et al. (1951). When nuclei were to be analyzed, suspensions in the storage buffer (0.01 M Tris/HCl, pH 7.5, containing 25% (wt/vol) glycerol and 1 mM $MgCl_2$) were made 0.3 M with respect to $HClO_4$ and sedimented at 2,000 *g* for 10 min. Pelleted nuclei or aliquots of chromatin preparations were analyzed for DNA, RNA, and protein by the methods of Spelsberg and Hnilica (1971) using the above analytical techniques.

Electron Microscopy

Pellets of nuclei were fixed with 2% glutaraldehyde, postfixed with osmium tetroxide, stained en bloc with uranyl acetate, dehydrated in ethanol and propylene oxide, and embedded in Araldite. After heat polymerization, 1.0–2.0-μm sections including a full thickness of pellet were examined by phase-contrast microscopy. Thin sections of selected areas were stained with uranyl acetate and lead citrate and examined in a Philips EM-300 electron microscope.

RESULTS

Structural Integrity and Purity of the Nuclei

Figs. 1 and 2 are electron micrographs of nuclei prepared by the four isolation methods. All preparations were relatively free of contamination, the most common inclusion in the hypertonic sucrose preparations (methods I and II) being rounded dense bodies, 1–2 μm in diameter and containing electron-lucent areas (Fig. 1 *c*). These structures frequently had ribosomes and membranous cytoplasmic components adherent to their surface and are thought to represent secretory granules from oviduct glandular cells. Nuclei prepared by the bulk method also contained occasional undisrupted cells with strips of intact muscle cells being most frequent. As expected, Triton X-100 almost completely removed the outer nuclear membrane whereas non-Triton-treated nuclei retained the outer membrane and attached ribosomes. Nuclei prepared in citric acid showed no intact cells but most nuclei had adherent tags of cytoplasmic membranes and ribosomes. On the other hand, preparation by the $MgCl_2$/citric acid method, which included a detergent treatment, contained no intact cells and only scant fragments of cytoplasmic debris.

The nuclear fine structure was best preserved by hypertonic sucrose particularly in the bulk method (method II). These nuclei exhibited condensed and noncondensed chromatin, and the nucleoli clearly exhibited both fibrillar and granular components. Nuclei prepared in citric acid (method III) were severely damaged with large irregular areas devoid of structure and although nuclei prepared in $MgCl_{22}$/citric acid were less damaged, their ultrastructure exhibited a distorted pattern of course reticulation formed by interanastomosing beaded strands of chromatin material (Figs. 2 *c* and 2 *d*).

Chemical Composition of the Nuclei

Table I A illustrates the total protein and RNA content of typical preparations of nuclei isolated by the various techniques. Nuclei prepared by

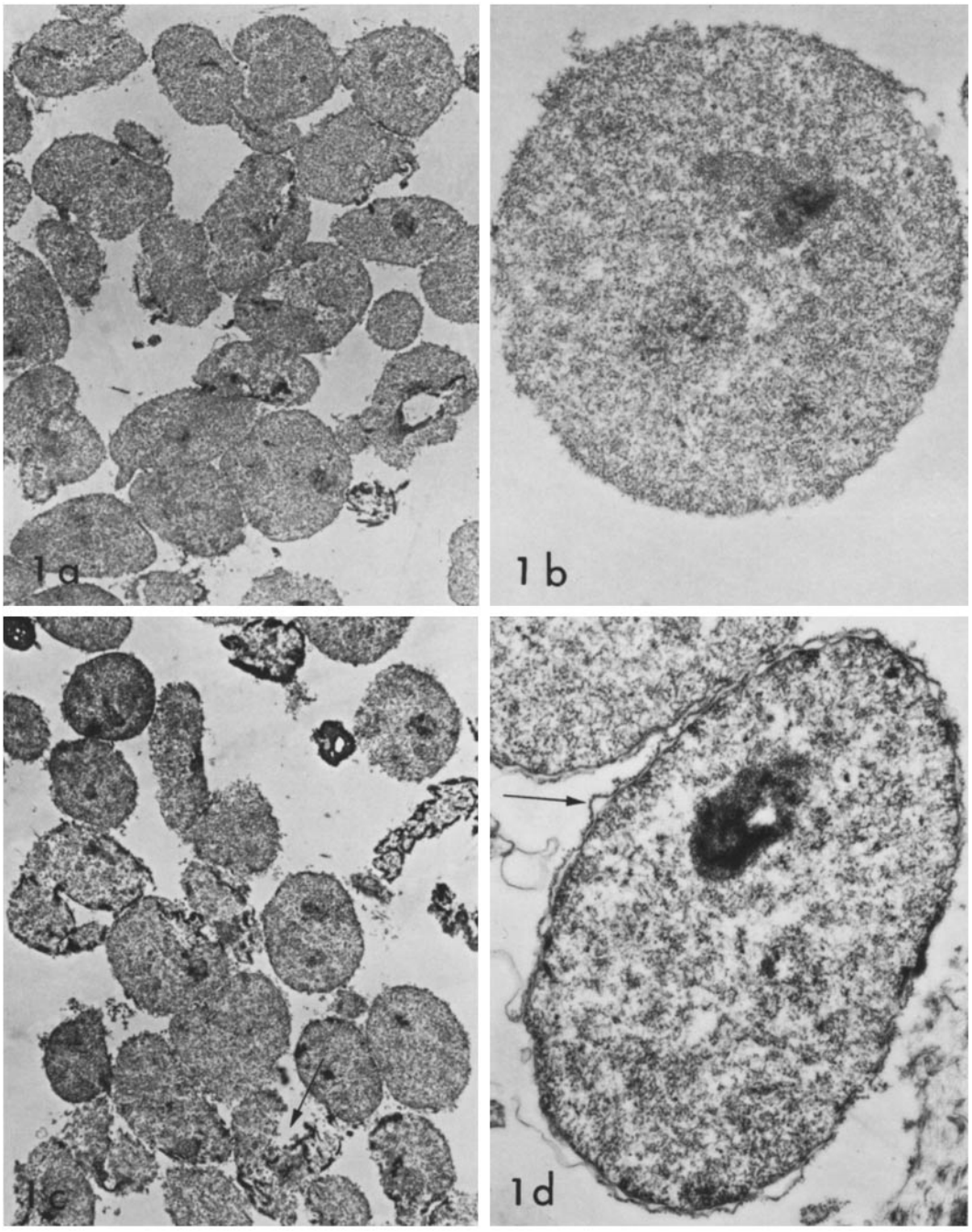

FIGURE 1 Electron micrographs of nuclei prepared by methods involving hypertonic sucrose. (*a* and *b*) Nuclei prepared by method I in the presence of Triton X-100. (*c*) Nuclei prepared by method II. The arrow points to a disrupted nucleus. Note also the dense bodies. (*d*) Nuclei prepared by method I in the absence of Triton X-100. *a* and *c*, × 3,500, *b* and *d*, × 15,000.

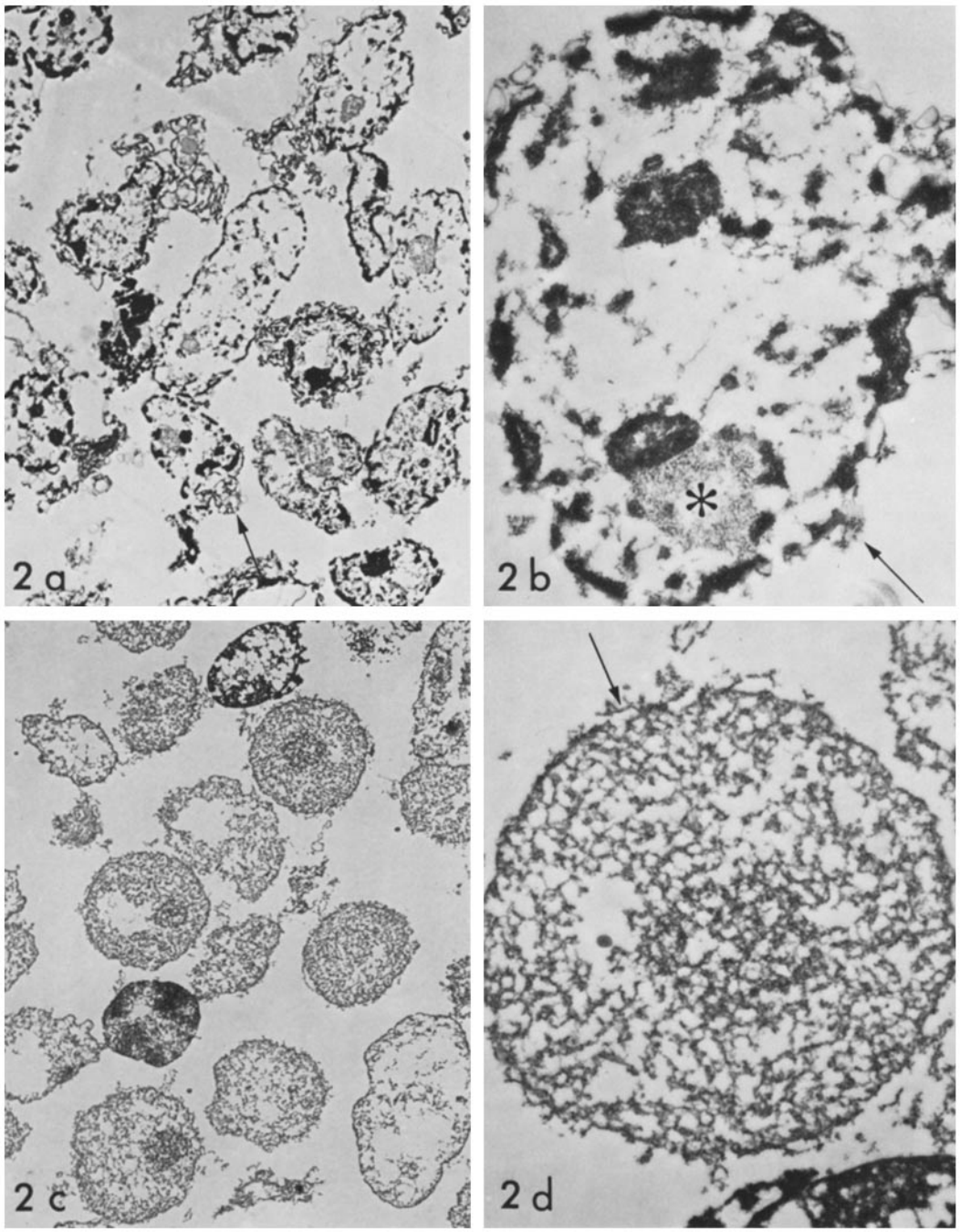

FIGURE 2 Electron micrographs of nuclei prepared by methods involving citric acid. (*a* and *b*) Nuclei prepared by method III. The arrows point to cytoplasmic fragments. (*c* and *d*) Nuclei prepared by method IV. The arrow points to small fragments of outer nuclear membrane. *a* and *c*, × 3,500, *b* and *d*, × 15,000.

TABLE I

The Levels of Protein, RNA, and Endogenous RNA Polymerase Activity in Nuclei Isolated by Various Methods

Nuclear isolation method	(A) Composition		(B) RNA polymerase activity (pmol [^{3}H]UMP incorporated/mg DNA)	
	Protein/DNA	RNA/DNA	Polymerase I (nucleolar)	Polymerase II (nucleoplasmic)
I With Triton X-100	2.05 ± 0.27	0.265 ± 0.014	0.87 ± 0.27	27.49 ± 3.76
I Without Triton X-100	2.47 ± 0.17	0.346 ± 0.019	3.43 ± 0.36	34.63 ± 0.85
II	2.53 ± 0.25	0.553 ± 0.056	10.22 ± 1.38	21.91 ± 0.20
III	2.73 ± 0.08	0.729 ± 0.081	0.000	2.00 ± 0.96
IV	2.18 ± 0.37	0.218 ± 0.012	0.79 ± 0.29	3.24 ± 0.24

Methods for the analysis of nuclear composition and the assay of endogenous RNA polymerase activities are described in the Methods section. The results show the mean and range of two determinations.

sedimentation through 2.0 M sucrose solution (method I) or in $MgCl_2$/citric acid (method IV) displayed the lowest protein and RNA content. The bulk preparative procedure (method II) and the citric acid method (III), which were shown by electron microscopy to yield slightly less pure preparations, gave somewhat higher protein and RNA values. The treatment of nuclei with Triton X-100 reduced the RNA and protein content.

Levels of Endogenous RNA Polymerase

The assay procedure for DNA-dependent RNA polymerase (see Methods section) was found to be dependent on the presence of all four nucleotides, DNA template, and divalent ions. The reaction was time dependent and its product was ribonucleic acid as judged by its susceptibility to ribonuclease and alkali hydrolysis. Under the assay conditions described, ribonuclease activity was minimal and RNA synthesis was linear. The low salt reactions, which were conducted in the presence of α-amanitin (Stirpe and Fiume, 1967; Kedinger et al., 1970) synthesized a ribosomal RNA (rRNA)-like product which had a U/G ratio of 0.75 characteristic of nucleolar RNA polymerase Reactions containing high salt but no α-amanitin synthesized DNA-like RNA which had a U/G ratio of 1.1 characteristic of nucleoplasmic RNA polymerase.

Table I B shows the analysis of nucleolar and nucleoplasmic polymerase activities in the purified nuclei. Nuclei isolated in 2.0 M sucrose (method I) displayed the highest polymerase activity but when the method included treatment with the neutral detergent, Triton X-100, activities were always decreased. Exposure of the nuclei to lower concentrations of sucrose (0.5 M or less) as in method II always caused a decrease in nucleoplasmic polymerase activity but had variable effects on the nucleolar enzyme. Preparation of nuclei in the presence of citric acid (methods III and IV) essentially abolished the activity of both polymerases.

Stability of RNA During Nuclear Isolation

In Fig. 3, the four methods of preparing nuclei have been compared using the retention and integrity of RNA as a criteria of the quality of the preparation. Oviducts were incubated in an in vitro system in the presence of tritiated uridine and Fig. 3 *a* shows the incorporation of the precursor into total tissue RNA. Peaks of labeling occur in the pre-rRNA and rRNA species and these are superimposed on a background of heterogeneous nuclear RNA (HnRNA). The identity of the pre-rRNA species has been confirmed by methylation studies and, in the very similar profile in the labeling of rat uterine RNA, the various species have been characterized by methylation, base ratio, decay, rate of synthesis, and intracellular location (Knowler and Smellie, 1971, 1973).

Citric acid is known to reduce nuclear fragility (Dounce, 1955) and Higashi et al. (1966) have reported that, in Walker carcinoma tissue, citric acid permitted the isolation of nuclei which retained high-molecular weight RNA despite considerable changes in their ultrastructure. Our results largely confirmed these observations and Fig. 3 *c* shows that the method of Higashi et al. (1966) (method III) preserved much of the nRNA. Results, however, were very variable; there was always some decay of HnRNA and some times this was much more marked than in Fig. 3 *c*.

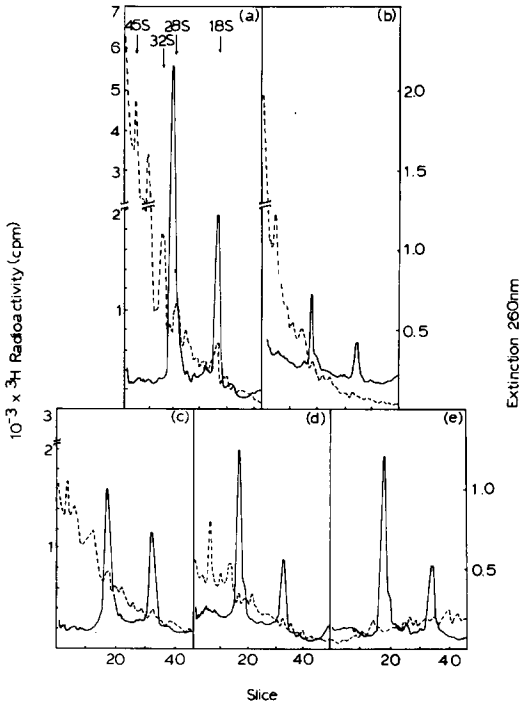

FIGURE 3 Retention of RNA during nuclear isolation. Excised chick oviducts were incubated in vitro in the presence of 25 μCi/ml of [5^{3}H]uridine as described in the Methods section. Nuclei were isolated by various methods and RNA immediately extracted. The extracted RNA was purified and subjected to electrophoresis on 2.7% polyacrylamide gels for 5 h at 5 mA per gel. (*a*) Total oviduct RNA. RNA isolated from (*b*) nuclei prepared by method IV; (*c*) nuclei prepared by method III; (*d*) nuclei prepared by method I; (*e*) nuclei prepared by method II; ——; extinction 260 nm: – – –, radioactivity per slice (cpm).

Fig. 3 *b* shows the profile of RNA species detected in nuclei prepared by the method of Knowler and Smellie (1973). This technique (method IV) also used citric acid together with $MgCl_2$ to protect the nuclei, had the added advantage of being very rapid, and incorporated a detergent treatment to remove any residue of the outer nuclear membrane not removed by acid. The preparation had a distribution of radioactivity in HnRNA very like that of total tissue, distinct peaks of 45S and 32S pre-rRNA but only very small peaks associated with the ribosomal subunit species. The extinction at 260 nm of 28S and 18S RNA was also markedly reduced.

The distribution of label in RNA after nuclei had been isolated in dense sucrose (method I) is illustrated in Fig. 3 *d*. Here, the pre-rRNA species were still strongly labeled but there was marked decay of HnRNA. When the nuclear preparation was entirely in dense sucrose solutions, the sugar seemed to exert some degree of protection since the preparation of these nuclei took 1½–2 h, much longer than the isolations involving more dilute sucrose solutions where, in the absence of citric acid, degradation was much more complete. This is illustrated in Fig. 3 *e*, showing the distribution of radioactivity in the RNA of nuclei isolated by a method in which the homogenization and detergent treatment steps were in 0.5 M sucrose (method II).

Effect of Nuclear Isolation on Chromatin Composition

Table II shows the analyses of the chromatins prepared from nuclei isolated by methods I–IV.

TABLE II

Chemical Composition and Template Capacity of Chromatin from Nuclei Isolated by Various Methods

Nuclear isolation method	Histone/DNA	Acidic protein/DNA	Percent open template (in DNA-dependent RNA synthesis)
I With Triton X-100	1.37 ± 0.03	1.07 ± 0.01	2.44 ± 0.02
I Without Triton X-100	1.30 ± 0.08	0.74 ± 0.01	1.74 ± 0.70
II	1.01 ± 0.04	0.85 ± 0.04	3.39 ± 0.08
IV	1.17 ± 0.04	2.13 ± 0.01	10.06 ± 0.23
IV	0.95 ± 0.01	1.34 ± 0.01	9.30 ± 0.15

Methods for analyzing chromatin and determining the template capacity of the isolated chromatin are described in the Methods section. The percentage of open template was calculated with pure DNA as a template representing 100% open template.

With the procedures involving hypertonic sucrose, the percent of the total DNA in the isolated chromatins which can serve as a template for the in vitro DNA-dependent RNA synthesis ranges around 2.5–5.0% of that transcribable in pure DNA. With the citric acid procedures, however, the percent of open template is much higher. Quantitative analysis of the histones suggests that a loss of histone may have occurred. More detailed studies of the histones by gel electrophoresis has disclosed the loss of the lysine-rich histones, especially the very lysine-rich histones F_1 of I_a and I_b (Fig. 4). The loss of lysine-rich histone from nuclei prepared in citric acid, particularly at pH values below 3.8, has been reported by Dounce et al., 1966 and MacGillivray et al. (1972) and adequately explains the increased template activity.

Table II also shows the level of acidic proteins and histones in the isolated chromatin, and this is complemented in Figs. 4 and 5 with qualitative analysis on polyacrylamide gels. Chromatin from nuclei isolated in hypertonic sucrose (methods I and II) contain approximately twice as much protein as DNA and of this slightly more than half is histone. Both catagories of protein give very clear reproducible patterns on polyacrylamide gels. The presence of Triton X-100 during the nuclear preparation reduced the recovery of chromatin acidic proteins and resulted in the loss of some low-molecular weight acidic protein bands (compare gels 1 and 2 in Fig. 5). Inclusion of citric acid in nuclear isolation procedures resulted in large losses of high-molecular weight acidic proteins.

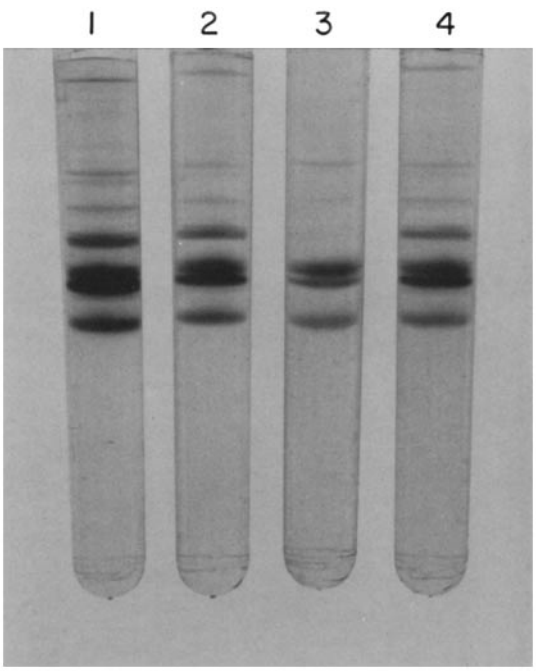

FIGURE 4 Polyacrylamide gel electrophoresis of total histones extracted from chromatin isolated from the various preparations of nuclei. 50 μg protein was applied per gel. Migration was from top (anode) to bottom (cathode). Histones of chromatin from nuclei prepared by method I with no Triton treatment (gel 1), method I with Triton treatment (gel 2), method IV (gel 3), and method II (gel 4).

Storage of Isolated Nuclei

When working with large numbers of samples, we have found it convenient to prepare nuclei from fresh tissue and store them for subsequent analysis. Fig. 6 shows the preservation of nucleolar and nucleoplasmic RNA polymerase in stored nuclei, and it is seen that in glycerol solutions at −20°C they retain 100% of their enzyme activity over 6 days while in other systems or at higher temperatures activity was markedly reduced. Further investigations have shown that nuclei stored in buffered glycerol retain most of their morphology, template capacity for RNA synthesis, and chemical composition as well as virtually all their RNA polymerase activities over a period of 3–6 months. Higher concentrations of glycerol were just as effective in preserving RNA polymerase but partially inhibited the enzyme activity upon assaying.

DISCUSSION

Chemical Composition and Morphology of Nuclei

Traditionally, the quality of a nuclear preparation is assessed by microscope examination and chemical composition and it is clear from the presented results that the two procedures are complementary. Thus, the chemical analysis (Table I) appears to correlate well with the electron microscope studies in that the greater the observed cytoplasmic contamination the greater was the protein and RNA content.

This is further borne out in one experiment in which 1.8 M sucrose was substituted for 2.0 M sucrose in the preparation of nuclei by method I.

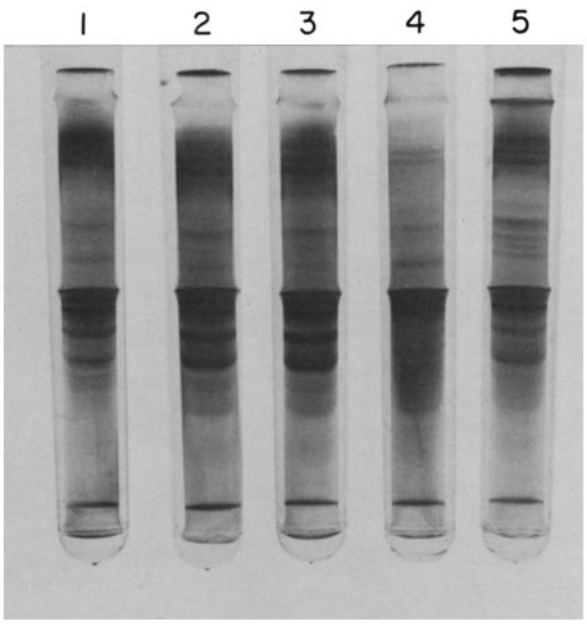

FIGURE 5 Polyacrylamide gel electrophoresis of total acidic proteins of chromatins from nuclei prepared by method I with Triton (gel 1), method I without Triton (gel 2), method I in 1.8 M sucrose without Triton (gel 3), method IV (gel 4), method II (gel 5). 150 μg of protein was applied per column. Migration was from top (cathode) to bottom (anode).

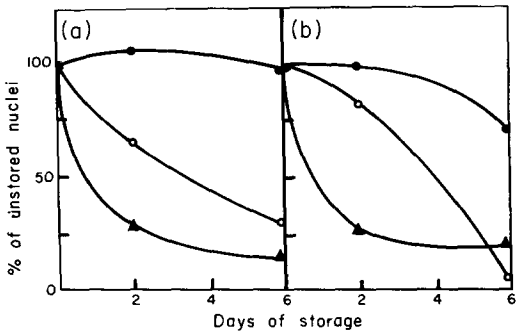

FIGURE 6 Levels of RNA polymerase II activity (assayed in high salt) in isolated nuclei after storage. Nuclei, isolated by method I (2 M sucrose method) were resuspended in (▲) 0.01 M Tris-HCl (pH 7.5), (○) 0.5 M sucrose in TKM buffer, or (●) 25% glycerol (vol/vol) in 0.01 M Tris-HCl (pH 7.5) + 0.001 M $MgCl_6$. The solutions were stored at −20°C (graph A) or at 4°C (graph B) for various periods of time. 50 μg DNA (as nuclei) were added to each reaction and the assays incubated at 15°C for 10 min.

Electron microscope examination of this preparation revealed significantly increased contamination with undisrupted epithelial cells and cytoplasmic debris, and nuclear analysis revealed twice as much protein and RNA per milligram DNA. The chromatin compositions of this preparation also revealed increases in the apparent histone (acid soluble) and acidic (acid insoluble) protein content but separations on polyacrylamide gels revealed identical histone patterns and only minor differences in the acidic protein patterns as compared with preparations from clean nuclei. Thus, contaminated preparations result in only small differences in qualitative compared with quantitative analysis (Fig. 5, gel 3). This fact ilustrates both the importance of starting with clean nuclei and the limitations of the qualitative techniques. Inclusion of Triton X-100 in the nuclear isolation medium results in the loss of a few acidic protein species, suggesting that they may not have been chromatin protein but associated with the outer nuclear membrane. It may be that the preparations are still contaminated with proteins from the inner nuclear membrane.

The nuclei with the best morphological appearance are not necessarily the ideal preparation. Nuclei prepared by the bulk method (method II) most closely resembled those of intact cells but the preparation was valueless in studying RNA species since the nuclear RNA was largely degraded. Conversely, nuclei prepared in citric acid (meth-

ods III and IV) had markedly altered ultrastructure but had retained most of their RNA in an undegraded state. It should also be mentioned that, although a chemical analysis is useful in assessing the purity of nuclear preparations, it cannot be determined to what extent lower levels of protein and RNA represent cleaner nuclei preparations as opposed to degradation or loss by diffusion and/or extraction of these macromolecules.

RNA Polymerase in Nuclei

One method of analyzing the degree of degradation or loss of proteins during the isolation of nuclei is to measure the activity of known nuclear enzymes: for example, DNA-dependent RNA polymerase. The results show that polymerase levels are very much higher when nuclei are isolated by methods using high-sucrose concentrations. The high osmolarity functions to preserve the integrity of the nuclear membranes and creates an environment in which nuclear constituents are less likely to leach into the isolation medium. In this respect, it is noteworthy that when Triton X-100 is used to strip the outer nuclear membrane or when part of the isolation procedure involves lower sucrose concentrations (method II) the nucleoplasmic polymerase activity is markedly reduced. The nucleolar enzyme activity is also reduced by addition of Triton X-100 but appears higher after isolation in lower sucrose concentrations. The reason for this rise is uncertain at the present time. That the polymerase activities are preserved in nuclei stored in glycerol has been reported elsewhere (Read and Mauritzen, 1970) and is useful for experiments involving multiple samples.

Stability of nRNA

In a nuclear preparation designed to investigate nRNA species, one would hope to retain the characteristic high-molcular weight RNA species in an undegraded state. At the same time, one would expect ribosomal 28S and 18S RNA to be a less conspicuous feature of the preparation. To what extent one would expect these two species to disappear, however, is not entirely clear. RNA prepared from purified HeLa cell nuclei contains some 28S RNA but very little of the 18S species it has been suggested that the absence of 18S RNA should be a criteria of purity (Penman, 1966). However, otherwise apparently clean preparations of nuclei from other cell types contain both rRNA species often detectable both by their extinction at 260 nm and the incorporation of radioactive precursor (Steele et al., 1965; Higashi et al., 1966; Knowler and Smellie, 1973). We have found that the nuclear isolation of Knowler and Smellie (1973) gives the least loss of the high-molecular weight RNA of all the procedures attempted in this laboratory and also yields the smallest residual peaks of 28S and 18S rRNA. Whether these small peaks reflect ribosomal contamination or nRNA is uncertain at present. Nuclei prepared in citric acid are not suitable for the other purposes investigated in this paper.

Received for publication 1 May 1973, and in revised form 10 August 1973.

REFERENCES

BLOBEL, G., and V. R. POTTER. 1966. *Science (Wash., D. C.).* **154**:1662.

BURGESS, R. R. 1969. *J. Biol. Chem.* **244**:6160.

BURTON, K. 1956. *Biochem. J.* **62**:315.

CERIOTTI, G. 1955. *J. Biol. Chem.* **214**:59.

CHAUVEAU, J., Y. MOULE, and C. H. ROUILLER. 1956. *Exp. Cell Res.* **11**:317.

DOUNCE, A. L. 1955. *In* The Nucleic Acids. E. Chargaff and J. N. Davidson, editors. Academic Press Inc., New York. **2**:93.

DOUNCE, A. L., F. SHERMAN, and M. MACKAY. 1966. *Arch. Biochem. Biophys.* **117**:550.

GLASSER, S. R., F. CHYTIL, and T. C. SPELSBERG. 1972. *Biochem. J.* **130**:947.

HIGASHI, K., K. S. NARAYANAN, H. R. ADAMS, and H. BUSCH. 1966. *Cancer Res.* **26**:1582.

JOEL, P. B., and D. D. HAGERMAN. 1969. *Biochim. Biophys. Acta.* **195**:328.

KEDINGER, C., M. GNIAZDOWSKI, J. L. MANDEL, F. GISSINGER, and P. CHAMBON. 1970. *Biochem. Biophys. Res. Commun.* **38**:165.

KNOWLER, J. T., and R. M. S. SMELLIE. 1971. *Biochem. J.* **125**:605.

KNOWLER, J. T., and R. M. S. SMELLIE. 1973. *Biochem. J.* **131**:689.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL. 1951. *J. Biol. Chem.* **193**:265.

MACGILLIVRAY, A. J., A. CAMERON, R. J. KRAUZE, D. RICKWOOD, and J. PAUL. 1972. *Biochim. Biophys. Acta.* **227**:384.

O'MALLEY, B. W., W. L. MCGUIRE, P. O. KOHLER, and S. G. KORENMAN. 1969. *Recent Prog. Horm. Res.* **25**:105.

PANYIM, S., and R. CHALKLEY. 1969. *Arch. Biochem. Biophys.* **130**:337.
PENMAN, S. 1966. *J. Mol. Biol.* **17**:117.
READ, R. S. D., and C. M. MAURITZEN. 1970. *Can. J. Biochem.* **48**:559.
ROEDER, R. G., and W. J. RUTTER. 1970. *Biochemistry*. **9**:2543.
SPELSBERG, T. C., and L. S. HNILICA. 1971. *Biochim. Biophys. Acta.* **228**:202.
SPELSBERG, T. C., A. W. STEGGLES, and B. W. O'MALLEY. 1971. *J. Biol. Chem.* **246**:4188.
STEELE, W. J., N. OKAMURA, and H. BUSCH. 1965. *J. Biol. Chem.* **240**:1742.
STIRPE, F., and L. FIUME. 1967. *Biochem. J.* **105**:779.
WIDNELL, C. C., and J. R. TATA. 1964. *Biochem. J.* **92**:313.
WILSON, E. M., and T. C. SPELSBERG. 1973. *Biochim. Biophys. Acta.* **322**:145.