# STUDY OF SOME FACTORS THAT AFFECT THE DISTRIBUTION OF NUCLEAR VOLUMES IN PREPARATIONS OF RAT LIVER NUCLEI[1]

ROBERTO UMAÑA[2,3]

*Institute of Nutrition of Central America and Panama, Guatemala, and Department of Biochemistry, School of Medicine of The University of San Carlos, Guatemala*

Received December 16, 1966

The effect of the homogenization procedure, the centrifugation scheme, and the composition of the suspension medium on the distribution of nuclear volumes has been studied.

It has been shown that the Waring Blendor not only destroys a greater number of the nuclei during homogenization, but also that this destruction is a selective one. At neutral pH values, no direct relationship appears to exist between the DNA content of the nuclei and their density. For this reason, purification in concentrated sucrose solutions produces a selective loss of the lighter nuclei, which includes small diploid stromal nuclei and some of the larger polyploid type of parenchymal nuclei.

The study of the effect of increasing the calcium and magnesium ion concentrations (from 0.001 to 0.005 *M*) on the nuclear distribution showed that these ions produce a selective shrinkage and condensation of the nuclei, probably through different mechanisms.

## Introduction

The preparations of isolated nuclei presently available are highly heterogeneous with respect to the size, form, density, and ploidy of their organelles, mainly because of the cellular heterogenity of the organs used as the source of nuclei.

The separation of the different nuclear types in sucrose density gradients depends on the different rates of migration of the nuclear species according to their volume, shape, and density. These properties, in turn, are intimately associated with the degree of condensation of the nucleoplasm, and the latter depends on the ionic composition and the pH of the medium (1–3). Previous work in our laboratory indicated that, besides the factors previously mentioned, the method of homogenization and the purification procedure may also alter the final distribution of certain types of nuclei in the preparation (4, 5). Therefore, it was felt that further efforts to fractionate nuclear preparations at neutral pH values would be greatly helped by a reevaluation and extension of knowledge about the effect of the media and the conditions of isolation on the distribution of nuclear volumes in the final preparation.

The present article describes a study of the effect of the method of homogenization, the pH, the purification procedure, and the more commonly employed concentrations of calcium and magnesium ions on the nuclear volume distribution.

[1]This work was supported by the United States Public Health Service, National Institute of Arthritis and Metabolic Diseases (grant AM-09126-01).

[2]The author gratefully acknowledges the technical assistance of Mr. Daniel Fernandez.

[3]Present address: Division of Human Genetics, School of Dentistry, University of Minnesota, Minneapolis, Minnesota 55455. Reprint requests should be directed to this address.

## Methods

The nuclei studied were isolated from the livers of female rats of the Sprague–Dawley strain weighing 200–250 g.

The effect of the method of homogenization on the distribution of the nuclear volumes was studied in nuclei isolated by differential centrifugation in dilute citric acid at pH 3.6 (6). Some of the preparations were homogenized in dilute citric acid (final pH of the homogenate, 3.6) with a Waring Blendor run at full speed (about 20,000 r.p.m.) for 3 minutes. Other preparations were homogenized in the same medium but with 5 strokes of the "L" pestle and 15 strokes of the "T" pestle in the ball-type homogenizer described by Dounce (1).

The effect of the pH of the medium on the distribution of nuclear volumes was studied in nuclei isolated by differential centrifugation in 0.44 *M* sucrose at pH 5.8 (6). The homogenates were made with the ball-type homogenizer as previously described, and no purification step by flotation was included. The final preparation was resuspended in 0.44 *M* sucrose with the ball-type homogenizer, and six equal aliquots were withdrawn. The nuclei in each aliquot were collected by high-speed centrifugation and were resuspended in 0.44 *M* sucrose with the pH adjusted to 6.0 (control), 5.5, 4.0, 3.5, 3.0, and 2.5. No studies were carried out between pH values of 5.5 and 4.0 because the agglutination of the nuclei in this pH range precluded their proper measurement.

To study the effect of the purification step on the nuclear volume distribution, some preparations of nuclei were isolated in 0.44 *M* sucrose at pH 5.8, and were purified by resuspension in a 2.2 *M* sucrose solution and centrifugation at 27,000 × *g* for 30 minutes. The final preparations were resuspended in 0.44 *M* sucrose at pH 2.5 and measured. These results were compared with the measurements obtained from the nuclei preparations isolated in 0.44 *M* sucrose at pH 5.8 by differential centrifugation alone and also resuspended in the same medium at pH 2.5.

The effect of increasing concentrations of calcium and magnesium ions on the distribution of nuclear volumes was studied in nuclei isolated in 0.44 *M* sucrose at pH 5.8 and purified with a solution of 2.2 *M* sucrose (5). The final nuclear pellet was suspended in a given volume of 0.44 *M* sucrose and six equal aliquots were withdrawn. After the collection of the nuclei by high-speed centrifugation, the first aliquot was resuspended in 0.44 *M* sucrose; the others were resuspended in the same solution containing increasing concentrations (1–5 m*M*) of either calcium or magnesium chloride.

The total number of nuclei was determined by direct counting in a Levy-type hemocytometer chamber as previously described (7); the pH of the acidified suspension of nuclei was about 2.5. The concentration of DNA was determined by the diphenylamine reaction of Dische described previously (7), using a standard solution of DNA extracted from rat liver nuclei, as described by Kay *et al.* (8).

The volume of the nuclei was calculated as a function of the diameter, assuming a perfect spherical shape. The diameters were directly measured with a Zeiss calibrated micrometer mounted on a Zeiss phase-contrast microscope.

The oculars, the objective, and the distance between the eye pieces were kept constant throughout the study. All of the measurements were made at room temperature by the same person, without any previous knowledge of the preparation being measured. Flattening of the nuclei by the pressure of the cover slip with the corresponding increase in the diameter of the organelles was not considered a problem since enough medium existed between the slip and the microscope slide to allow all the nuclei to roll freely in the microscopic field. The microscopic fields were randomly chosen and all the nuclei in each field were measured, an average of 400 measurements being taken for each preparation. The small, pear-shaped nuclei were measured along their longest axis, the spherical nuclei were measured along the "equator", and the elongated nuclei were measured along both axes and the average used as the diameter. The results were arranged in groups whose diameters differed by 2 $\mu$.

## Results

Microscopically, it was possible to differentiate three main types of nuclei. One type consisted of small, pear-shaped nuclei with diameters ranging from 4 to 6 $\mu$. Another type consisted of large, spherical nuclei with diameters ranging from 8 to 14 $\mu$. The last group consisted of very elongated nuclei with the longest axis measuring from 8 to 12 $\mu$ and the smaller axis about 4 $\mu$. Because the frequency distribution of each group has been expressed in terms of percentage, it is necessary to bear in mind that a decrease or increase in any of the categories will be reflected in a proportional change in the other groups. Therefore, if in the phase of a decrease in the proportion of one of the groups, one or several of the other groups fail to increase or do not increase in the same proportion as the others, it will be necessary to assume that a certain amount of destruction or loss has occurred, upsetting the rise that otherwise would have taken place.

The effect of change in the pH of the medium can be seen in Table I. It is evident from this table that the change in pH produced a progressive two-phase effect. In the first phase, encompassing pH 6.0–4.0, there was a marked condensation and increased granularity of the nucleoplasm accompanied by a shrinking of the nuclei. The net effect was an accumulation of nuclei 6 $\mu$ in diameter, a drastic reduction in the proportion of nuclei 10 $\mu$ in diameter, and a complete disappearance of the largest nuclei. The average volume decreased from 222 to 176 $\mu^3$ (−21%). In the second phase, encompassing pH 3.5–2.5, there was a progressive swelling and a reduction in the nucleoplasm granularity and condensation. At pH 2.5, the nuclei were redistributed into two well-defined peaks, each one comprising about 50% of the overall population. The average volume of the nuclei increased from the previous minimum of 176 to a maximum of 391 $\mu^3$. The average volume for the nuclei in the first peak (nuclei 4–8 $\mu$ in diameter) was 145 $\mu^3$, and the average volume for the nuclei in the second peak (10–14 $\mu$ in diameter) was 619 $\mu^3$, in good agreement with the data previously published by Falzone *et al.* (9), and Santen (10).

The effect of the use of the Waring Blendor for homogenization is presented

in Table II. From this table, it can be seen that the Blendor produced a certain amount of destruction of the nuclei 4 and 6 $\mu$ in diameter and considerable destruction of the nuclei with diameters of 8 and 10 $\mu$. This reduction is reflected in the high proportion of larger nuclei (12 and 14 $\mu$ in diameter). The average volume of the nuclei increased about 10% which, according to the mean values presented in the table, most probably is nonsignificant. The

TABLE I

Effect of the pH of the medium on the distribution of nuclear volumes*

| | Nuclear diameter ($\mu$) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 4 | 6 | 8 | 10 | 12 | 14 | Mean volume ($\mu^3$) |
| pH | % distribution of nuclear volumes† | | | | | | |
| 6.0 | 14.1 | 25.3 | 50.1 | 9.8 | 0.3 | 0.0 | 222 |
| 5.5 | 13.5 | 25.1 | 53.9 | 7.3 | 0.0 | 0.0 | 215 |
| Δ%‡ | −4.2 | −0.8 | +7.6 | −25.5 | −100.0 | 0.0 | −3.1 |
| 4.0 | 15.1 | 41.7 | 40.0 | 3.1 | 0.0 | 0.0 | 176 |
| Δ% | +7.1 | +64.8 | −20.1 | −68.4 | −100.0 | 0.0 | −20.7 |
| 3.5 | 11.7 | 31.7 | 45.1 | 11.3 | 0.0 | 0.0 | 220 |
| Δ% | −17.0 | +25.3 | −9.9 | +15.3 | −100.0 | 0.0 | −0.9 |
| 3.0 | 14.6 | 24.6 | 30.9 | 26.7 | 2.3 | 0.6 | 285 |
| Δ% | +3.5 | −2.8 | −38.3 | +172.4 | +666.7 | | +28.4 |
| 2.5 | 9.9 | 22.2 | 14.6 | 43.9 | 7.5 | 1.8 | 391 |
| Δ% | −29.8 | −12.2 | −70.8 | +347.9 | +2400.0 | | +76.1 |

*Nuclei isolated by differential centrifugation in 0.44 *M* sucrose at pH 5.8 without using the purification step in 2.2 *M* sucrose.
†Each value is the average of two preparations.
‡The value of Δ% was calculated as $\Delta\% = 100(\bar{X}_e - \bar{X}_c)/\bar{X}_c$, where $\bar{X}_c$ = average value at pH 6.0 and $\bar{X}_e$ = average value at the pH value considered.

TABLE II

Effect of the method of homogenization on the distribution of nuclear volumes*

| | Nuclear diameter ($\mu$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 4 | 6 | 8 | 10 | 12 | 14 | Mean volume ($\mu^3$) | Mean DNA content ($\mu\mu$g/nucleus) |
| Preparation No. | % distribution of nuclear volumes† | | | | | | | |
| | Ball-type homogenizer | | | | | | | |
| 1 | 3.2 | 22.5 | 11.9 | 40.8 | 19.1 | 2.3 | 478 | 11.0 |
| 2 | 5.9 | 19.3 | 25.6 | 30.0 | 14.3 | 4.9 | 462 | 11.3 |
| 3 | 12.9 | 29.2 | 27.9 | 25.2 | 4.7 | 0.0 | 287 | 11.3 |
| 4 | 10.6 | 19.6 | 7.2 | 27.6 | 24.8 | 10.1 | 558 | 7.5 |
| 5 | 18.0 | 14.0 | 14.0 | 34.0 | 11.0 | 8.0 | 451 | — |
| Average | 10.1 | 20.9 | 17.3 | 31.5 | 14.8 | 5.1 | 447 | 10.3 |
| | Waring Blendor | | | | | | | |
| 1 | 18.3 | 18.3 | 10.9 | 20.0 | 23.1 | 9.4 | 505 | 7.6 |
| 2 | 4.7 | 22.4 | 16.3 | 27.8 | 23.4 | 4.8 | 487 | 7.0 |
| Average | 11.5 | 20.3 | 13.6 | 23.9 | 23.2 | 7.1 | 496 | 7.3 |
| Δ%‡ | +13.9 | −2.9 | −21.4 | −24.1 | +56.7 | +39.2 | +10.9 | −29.1 |

*Nuclei isolated in 0.44 *M* sucrose at pH 2.5.
†Each value is the average of two preparations.
‡Δ% calculated as in Table I. $\bar{X}_c$ = average obtained with the ball-type homogenizer and $\bar{X}_e$ = average obtained with the Waring Blendor.

change in the average DNA content from a modal value of 11–7.3 $\mu\mu$g per nucleus suggests that there might have been a selective destruction of polyploid-type nuclei.

Table III shows the effect on the distribution of nuclear volumes of the use of 2.2 *M* sucrose for purification. This purification step, under our experimental conditions, produced a considerable loss of nuclei 4, 6, and 10 $\mu$ in diameter, and most probably a slight loss of nuclei 8 $\mu$ in diameter. Because of the proportional increase in the larger nuclei, the average volume of the nuclei in this preparation rose about 30%, from 392 to 511 $\mu^3$.

TABLE III

Effect of the purification step in 2.2 *M* sucrose on the distribution of nuclear volumes*

| Preparation No. | Nuclear diameter ($\mu$) | | | | | | Mean volume ($\mu^3$) |
|---|---|---|---|---|---|---|---|
| | 4 | 6 | 8 | 10 | 12 | 14 | |
| | % distribution of nuclear volumes† | | | | | | |
| | Without purification step in 2.2 *M* sucrose | | | | | | |
| 1 | 9.8 | 21.6 | 18.0 | 44.7 | 5.5 | 0.4 | 366 |
| 2 | 10.0 | 22.8 | 11.2 | 43.2 | 9.6 | 3.2 | 418 |
| Average | 9.9 | 22.2 | 14.6 | 43.9 | 7.5 | 1.8 | 392 |
| | With purification step in 2.2 *M* sucrose | | | | | | |
| 1 | 4.7 | 17.7 | 19.3 | 45.3 | 8.8 | 4.2 | 451 |
| 2 | 0.6 | 13.1 | 21.0 | 32.2 | 26.3 | 6.5 | 571 |
| Average | 2.6 | 15.4 | 20.1 | 38.7 | 17.5 | 5.3 | 511 |
| Δ%‡ | −73.7 | −30.6 | +37.7 | −11.8 | +133.3 | +194.4 | +30.3 |

*Nuclei isolated in 0.44 *M* sucrose at pH 2.5.
†Each value is the average of two preparations.
‡Δ% calculated as in Table I.

TABLE IV

Effect of increasing calcium concentrations on the distribution of nuclear volumes*

| Concn. (m*M*) | Nuclear diameter ($\mu$) | | | | | | Mean volume ($\mu^3$) |
|---|---|---|---|---|---|---|---|
| | 4 | 6 | 8 | 10 | 12 | 14 | |
| | % distribution of nuclear volumes† | | | | | | |
| 0 | 3.7 | 17.4 | 32.9 | 28.1 | 14.0 | 3.3 | 430 |
| 1 | 2.9 | 28.3 | 45.0 | 19.5 | 4.1 | 0.0 | 293 |
| Δ%‡ | −21.6 | +62.6 | +36.8 | −30.6 | −70.7 | −100.0 | −31.9 |
| 2 | 2.2 | 21.7 | 55.7 | 19.2 | 1.0 | 0.0 | 284 |
| Δ% | −40.5 | +24.7 | +69.3 | −31.7 | −92.8 | −100.0 | −33.9 |
| 3 | 3.6 | 27.0 | 52.6 | 16.2 | 0.5 | 0.0 | 262 |
| Δ% | −2.7 | +55.2 | +59.9 | −42.3 | −96.4 | −100.0 | −39.1 |
| 4 | 2.5 | 41.5 | 46.6 | 10.6 | 0.0 | 0.0 | 228 |
| Δ% | −32.4 | +138.5 | +41.6 | −62.3 | −100.0 | −100.0 | −46.9 |
| 5 | 8.0 | 41.9 | 44.8 | 4.6 | 0.0 | 0.0 | 194 |
| Δ% | +116.2 | +140.8 | +36.2 | −83.6 | −100.0 | −100.0 | −54.9 |

*Nuclei isolated in 0.44 *M* sucrose at pH 5.8 and purified with 2.2 *M* sucrose.
†Each value is the average of two preparations.
‡Δ% calculated as in Table I.

The effect of increasing the concentration of calcium ions in the medium is presented in Table IV. Concentrations up to 4 m*M* produced, in the smaller nuclei (4 $\mu$ in diameter), what most probably would be nonsignificant changes in a statistical study. The larger nuclei showed a high degree of granulation, condensation, and shrinkage. The greatest shrinkage was observed at concentrations of 5 m*M*, with a reduction in the average nuclear volume of about 55% (from 430 to 194 $\mu^3$).

TABLE V

Effect on increasing magnesium concentrations on the distribution of nuclear volumes*

| Concn. (m*M*) | Nuclear diameter ($\mu$) | | | | | | Mean volume ($\mu^3$) |
|---|---|---|---|---|---|---|---|
| | 4 | 6 | 8 | 10 | 12 | 14 | |
| | % distribution of nuclear volumes† | | | | | | |
| 0 | 3.7 | 14.4 | 32.9 | 28.1 | 14.0 | 3.3 | 430 |
| 1 | 1.3 | 22.1 | 29.8 | 42.7 | 3.9 | 0.0 | 364 |
| Δ%‡ | −64.9 | +53.5 | −9.4 | +51.9 | −72.1 | −100.0 | −15.3 |
| 2 | 9.5 | 35.6 | 47.9 | 6.4 | 0.0 | 0.0 | 205 |
| Δ% | +156.7 | +147.2 | +45.6 | −77.2 | −100.0 | −100.0 | −52.3 |
| 3 | 13.4 | 29.5 | 56.9 | 1.3 | 0.0 | 0.0 | 197 |
| Δ% | +262.2 | +104.9 | +72.9 | −95.4 | −100.0 | −100.0 | −54.2 |
| 4 | 8.2 | 33.1 | 54.1 | 4.1 | 0.0 | 0.0 | 207 |
| Δ% | +121.6 | +129.9 | +64.4 | −85.4 | −100.0 | −100.0 | −51.9 |
| 5 | 14.7 | 32.8 | 50.2 | 2.1 | 0.0 | 0.0 | 187 |
| Δ% | +297.3 | +127.8 | +52.6 | −92.5 | −100.0 | −100.0 | −56.5 |

*Nuclei isolated in 0.44 *M* sucrose at pH 5.8 and purified with 2.2 *M* sucrose.
†Each value is the average of two preparations.
‡Δ% calculated as in Table I.

Table V shows the effect of increasing concentrations of magnesium ions on the distribution of nuclear volumes. The effect of this cation was also manifested as a progressive and selective shrinkage. At concentrations of 1 m*M*, the largest nuclei were more affected than the smaller, as judged by the different degree of change in the proportion of the nuclear types. When concentrations of 2 m*M* were reached, it appeared that maximum shrinkage had been achieved, in contrast with the effect of calcium ions, which shrank the nuclei maximally at concentrations of 5 m*M*. Interestingly enough, this magnesium ion concentration reduced the average volume in the same proportion (about −54%) as did calcium ions in concentrations of 5 m*M*.

## Discussion

In the present studies, a 0.44 *M* sucrose solution was used as the basic medium for the isolation of nuclei, to protect not only their structure (2, 3), but also to avoid osmotic disruption of other intracellular organelles that might liberate soluble hydrolytic enzymes capable of interfering with the measurement of nuclear sizes (11).

For some time it has been clear that motor-driven homogenizers of the

Waring Blendor type destroy a higher proportion of nuclei during homogenization than the coaxial-type homogenizers, especially when manually driven (1, 2, 6, 12). So far, the possibility of selective destruction of some types of nuclei has not been considered a great problem, but now that the feasibility of in vitro studies has made the isolation of homogeneous populations of nuclei desirable, the problem has to be evaluated. The results presented in Table II certainly indicate that nuclei of 6, 8, and 10 $\mu$ in diameter are more susceptible to mechanical breakage than the smallest and the largest nuclei. If, as suggested by Falzone *et al.* (9), the 6- and 8-$\mu$ nuclei are diploids of stromal origin, and the 10-$\mu$ nuclei are polyploids of parenchymal origin, it follows that the nuclear fragility does not directly depend on the amount of DNA and that the cellular origin does not influence this property either. It is probable that pathological conditions will alter, among other things, the fragility of some types of nuclei; therefore the results of the comparison of average compositions or metabolic activities between pathological nuclei and normal controls might be greatly influenced by selection of nuclei according to fragility during homogenization. The same argument should hold true for the comparison of results obtained by different investigators. Despite the fact that coaxial homogenizers are in general gentler than the Waring Blendor type, it might be wise to control this type of artifact, especially in the case of motor-driven coaxial homogenizers, to avoid possible discrepancies in the results.

The choice of isolation media has been, and most probably will continue to be, empirically made. The isolation of nuclei in dilute sucrose solutions requires the addition of very small amounts of divalent cations (1–5 m$M$ calcium or magnesium chloride), or the acidification of the medium by the addition of small amounts of acid. The most commonly used pH values range from 5.8 to 7.0 and from 4.0 to 2.5, depending on the type of study planned. Low pH values permit the isolation of highly pure nuclei, but due to extraction and denaturation of proteins, the organelles are not suitable for composition or metabolic studies (1, 2). The pH value of 5.8 has been used extensively in our studies because it has been shown that at this value there is a minimum of proteolytic activity (6).

Changes in the pH or in the concentration of calcium or magnesium ions have been shown to increase the condensation of the nucleoplasm as judged by an increased granularity and a reduced volume of the nuclei (1–3), this phenomenon being dependent on the integrity of DNA (3, 13, 14). Anderson and Wilbur (3) studied the effect of changes in the pH of the medium from 8.9 to 5.12 in the presence of low concentrations of potassium ions (0.023 $M$) in a hypotonic sucrose solution (0.145 $M$). In our studies, the range studied has been extended to pH 2.5, where several authors have been able to fractionate the nuclei according to their ploidy (9, 10), and under conditions in which the interference of monovalent cations and hypotonicity of the medium have been avoided. From our data (Table I), it is evident that an increase in the concentration of hydrogen ions does not affect all the nuclei to the same extent,

but up to pH 4.0 produces a selective and progressive shrinkage of the larger nuclei. Since the larger nuclei appear to be mainly polyploids (9, 10), this type of response would support the hypothesis that the shrinkage phenomenon produced by the hydrogen ions depends mainly on the amount of DNA present in the nucleus. The swelling of the nuclei when the pH is lowered from 4.0 to 2.5 resembles the "reversal of charge" phenomenon described by Anderson and Wilbur (3); therefore, it might be possible to speculate that the swelling is due to an increase in repulsive forces within the nucleus produced by having all the proteins below their isoelectric point, and to a greater hydration of DNA permitted by the partial extraction of histones and other nuclear proteins produced by the low pH of the medium.

The distribution of nuclei at pH 6.0 (Table I) did not show a clear separation between small, stromal nuclei and large, parenchymal nuclei. Fisher *et al.* (15), by sucrose gradient centrifugation, separated rat liver nuclei at neutral pH values into three fractions. The lighter fraction had an average DNA phosphorus content of $0.67 \pm 0.17$ $\mu\mu$g per nucleus; the intermediate fraction contained $0.84 \pm 0.18$ $\mu\mu$g per nucleus, and the heavier fraction contained $0.93 \pm 0.14$ $\mu\mu$g per nucleus. If it is accepted that the lighter fraction is mainly of diploid nuclei, the theoretical value for tetraploids and octaploids should be 1.34 and 2.68 $\mu\mu$g of DNA phosphorus per nucleus, respectively. Since neither the intermediate-density nor the higher density bands reached the theoretical values, and since there is no statistical significance ($N = 6$) between the mean values of the lighter density and the intermediate-density fractions and between those of the intermediate-density and the higher density fractions, it follows that Fisher's fractionation did not achieve a complete separation of the nuclei according to ploidy, even though it separated them according to densities. Each of the fractions (intermediate-density and higher density) most probably consisted of a mixture of diploid and polyploid.

Based on this interpretation of Fisher's fractionation and on the selective way in which nuclei appear to be lost during purification in 2.2 *M* sucrose, it is possible to hypothesize that nuclear density at neutral pH values is not a unique function of the DNA content but, most probably, depends on their protein content as well. At low pH values, the condensation of the nucleoplasm and the loss of some protein might make the nuclear density depend mainly on the DNA content, allowing for the fractionation achieved by Falzone *et al.* (9) and by Santen (10).

Concentrations of calcium and magnesium ions ranging from 0.001 to 0.005 *M* are most commonly used for the isolation of nuclei in sucrose solutions at neutral pH values. Anderson and Wilbur (3) studied, among other concentrations, the effect of concentrations of 0.001 and 0.010 *M*, and concluded from their experiments that the changes in condensation and volume involved the greater proportion of the nuclei and not a single size range, and that the effect of calcium ions was different from that of magnesium ions. Naora *et al.* (16) have explained the latter observation by showing that calcium combines

mainly with the nuclear proteins whereas magnesium interacts mainly with the phosphate groups of DNA. Our results support the difference in the effect of calcium and magnesium ions, but since the increments in ion concentration were smaller (1–5 m$M$) than those used by Anderson and Wilbur (1–10 m$M$), we have been able to show that the larger nuclei are more susceptible to the effect of these ions than the smaller nuclei. The fact that the smaller average volume was obtained at magnesium ion concentrations of 2 m$M$ might indicate that the saturation point of the DNA phosphate groups had been reached; the necessity of increasing the concentration of calcium ions to 5 m$M$ to obtain the same shrinking effect might be due to the need for larger amounts of calcium ions to saturate the more abundant nuclear proteins. The higher susceptibility of larger nuclei to either cation can be explained by their higher DNA content in the case of magnesium ions and their higher protein content in the case of calcium ions.

The fact that small changes in the conditions for isolation selectively affect one type of nuclei with respect to the others further warns us against a simple interpretation of average measurements of nuclear volumes and against the temptation to correlate directly those measurements with the degree of ploidy.

## References

1. A. L. DOUNCE. *In* The nucleic acids. Vol. II: *Edited by* E. Chargaff and J. N. Davidson. Academic Press, Inc., New York. 1955. p. 93.
2. V. G. ALLFREY. *In* The cell. Vol. I. *Edited by* J. Brachet and A. E. Mirsky. Academic Press, Inc., New York. 1959. p. 193.
3. N. G. ANDERSON and K. M. WILBUR. J. Gen. Physiol. **35**, 781 (1952).
4. R. UMAÑA. Can. J. Biochem. **43**, 125 (1965).
5. R. UMAÑA. Can. J. Biochem. **44**, 187 (1966).
6. A. L. DOUNCE and R. UMAÑA. Biochemistry, **1**, 811 (1962).
7. R. UMAÑA. J. Nutr. **85**, 169 (1965).
8. E. R. M. KAY, N. S. SIMMONS, and A. L. DOUNCE. J. Am. Chem. Soc. **74**, 1724 (1952).
9. J. A. FALZONE, C. H. BARROWS, and M. J. YIENGST. Exptl. Cell. Res. **26**, 552 (1962).
10. R. J. SANTEN. Exptl. Cell. Res. **40**, 413 (1965).
11. N. G. ANDERSON. Exptl. Cell. Res. **5**, 361 (1953).
12. H. LØVTRUP-REIN and B. S. MCEWEN. J. Cell. Biol. **30**, 405 (1966).
13. N. G. ANDERSON and C. B. NORRIS. Exptl. Cell Res. **19**, 605 (1960).
14. J. RIS, A. E. MIRSKY, and V. G. ALLFREY. J. Gen. Physiol. **32**, 489 (1949).
15. R. F. FISHER, D. J. HOLBROOK, and J. L. IRVIN. J. Cell. Biol. **17**, 231 (1963).
16. H. NAORA, A. E. MIRSKY, and V. G. ALLFREY. J. Gen. Physiol. **44**, 713 (1961).