

STUDIES ON ISOLATED CELL COMPONENTS

IV. THE EFFECT OF VARIOUS SOLUTIONS ON THE ISOLATED RAT LIVER NUCLEUS*

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PLATE 2

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There are as yet no satisfactory substitutes for intracellular fluids suitable for the prolonged maintenance of cell components *in vitro*. That such media are a prerequisite for all studies on homogenates and isolated cell components is apparent and has been repeatedly emphasized (1-4). The difficulty in devising media lies of course in our inability to obtain cytoplasmic fluid for analysis without initiating injury reactions which alter the normal state of the cytoplasm. The alternative is to start with such data as are available on the composition of cytoplasm and to proceed empirically in devising appropriate solutions. This general approach has been used in working out solutions for the isolation of rat liver nuclei; and a buffered salt-sucrose mixture has been found in which nuclei are optically similar to those within intact liver cells (4).

These initial investigations were concerned primarily with problems of nuclear isolation; and further study of the action of inorganic substances on the isolated nucleus is needed to provide a basis for the inclusion or exclusion of specific ions in media for mammalian nuclei and also for a clarification of properties of the nuclear substance. These problems are considered in the present study which deals with the effects of inorganic ions and pH on the isolated rat liver nucleus. In addition to a study of specific ion effects, the osmotic behavior of isolated nuclei has been investigated.

Numerous studies have been concerned with the effects of inorganic solutions on invertebrate, vertebrate, and plant nuclei. These include the effects of salts on nuclear structure (5-15), changes in structure produced by acid and alkaline solutions (8, 16-18), and osmotic changes (19-21). However, no systematic study of the effects of electrolytes, pH, and tonicity appears to have been made on mammalian nuclei. Of the previous studies that of Ris and

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Mirsky (14) is most closely related to the present investigation and the experiments reported here are in large measure a continuation of their work.

Methods

Rat liver nuclei were isolated in phosphate-bicarbonate-sucrose solutions by differential centrifugation essentially as previously described (4). A few experiments were also carried out on crude nuclear preparations sedimented directly from the original homogenate in order to eliminate the variation in tonicity encountered in the differential centrifugal procedure. The nuclei showed the same behavior with both methods and the more highly purified preparation was used as routine.

Nuclei were prepared and maintained at 0°C. during the course of the experiments. Observations were made on uncooled slides at room temperature (usually 22–24°C. though occasionally higher) within a period of 3 hours after the liver was removed from the rat. In studying the effects of a particular electrolyte on nuclear volume a complete series of concentrations was studied on each nuclear preparation. In order to cancel out any change in response with time after isolation the order in which the concentrations were studied was reversed in different preparations. The complete series was repeated on preparations from at least 3 other animals and the results averaged. From 46 to 56 nuclei were measured at each concentration.

For observation and photomicrographs several drops of a nuclear preparation were placed between slide and coverslip to give a layer whose thickness was several times the nuclear diameter. The solution to be studied was then introduced at one side of the coverslip while fluid was carefully withdrawn with absorbent paper from the other side. Observations and measurements were confined to nuclei close to the region in which the experimental solution was introduced. Preliminary studies showed that the nuclear volume would reach equilibrium within 3 minutes after the introduction of the experimental solution with no further change during the course of 15 minutes except with those solutions which caused nuclear disintegration. Except in the preliminary series shown in Table I, nuclear volumes were from phase contrast photomicrographs enlarged to 8 × 10 inches on very high contrast photographic paper. Only those nuclei in sharp focus were measured. Approximately 900 photomicrographs were required for the measurements reported.

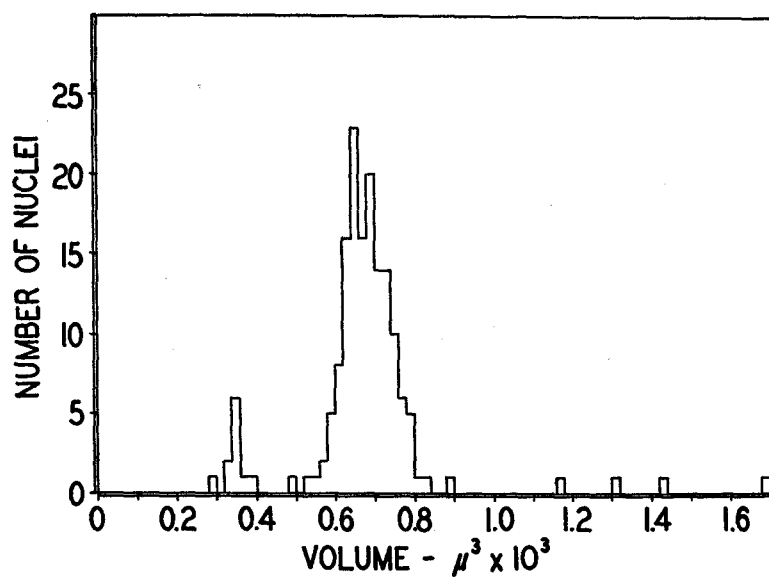
All solutions were made with glass-distilled water. The pH of the buffered salt-sucrose isolation mixture was 7.1. Adult rats of the Osborne-Mendel strain were used for all studies.

RESULTS

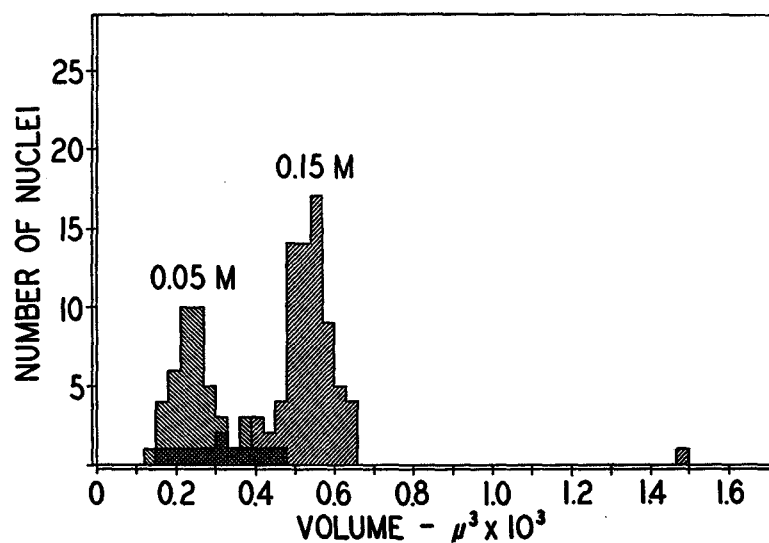
Frequency Distribution of Nuclear Size

Before presenting the experimental studies on volume changes in isolated nuclei, consideration will first be given to size distribution of nuclei obtained by differential centrifugation. Measurements were made from enlargements of nuclei photographed immediately after isolation in a buffered salt-sucrose solution (solution I: 0.0094 M KH_2PO_4 ; 0.0125 M K_2HPO_4 ; 0.0015 M NaHCO_3 ; 0.145 M sucrose).

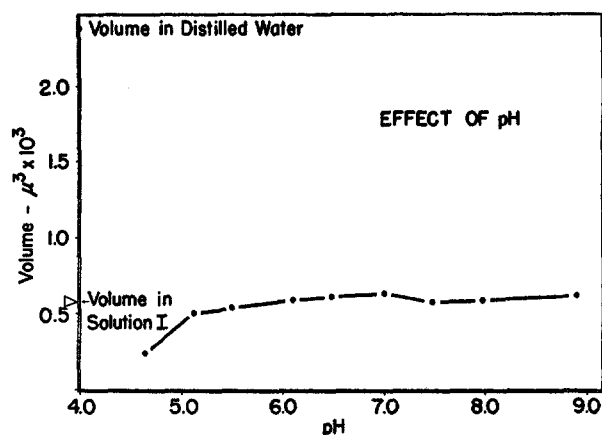
The frequency distribution of nuclear volumes of 162 nuclei from 4 adult rats is given in Text-fig. 1. The greater proportion (86 per cent) fell within a relatively



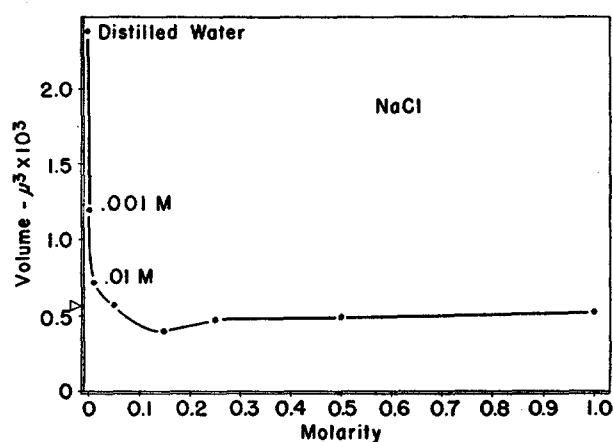
TEXT-FIG. 1. Size distribution of rat liver nuclei isolated in salt-sucrose buffer solution, pH 7.1. Total number of nuclei 162.



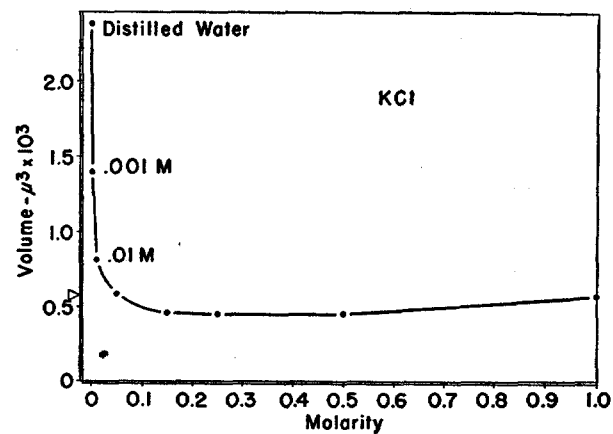
TEXT-FIG. 2. Size distributions of rat liver nuclei in 0.05 M and 0.15 M CaCl_2 following isolation in salt-sucrose buffer solution. From Text-fig. 1 it is evident that the experimental solutions affect the major portion of the nuclei as shown by the distribution pattern.



TEXT-FIG. 3

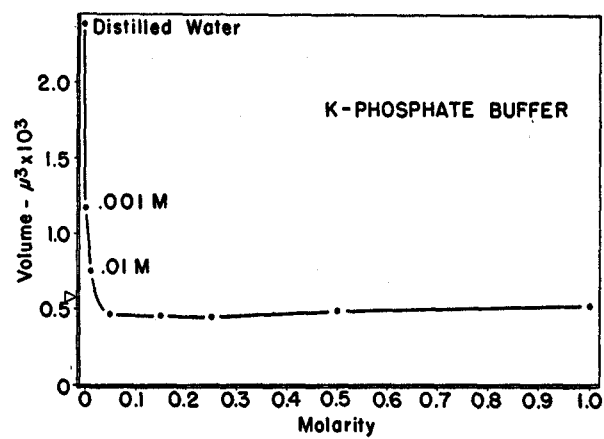


TEXT-FIG. 4

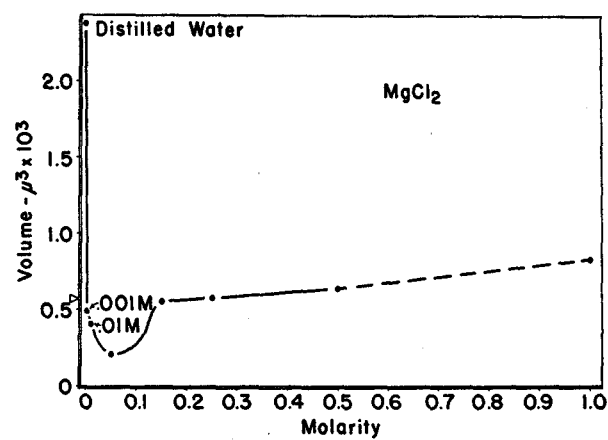


TEXT-FIG. 5

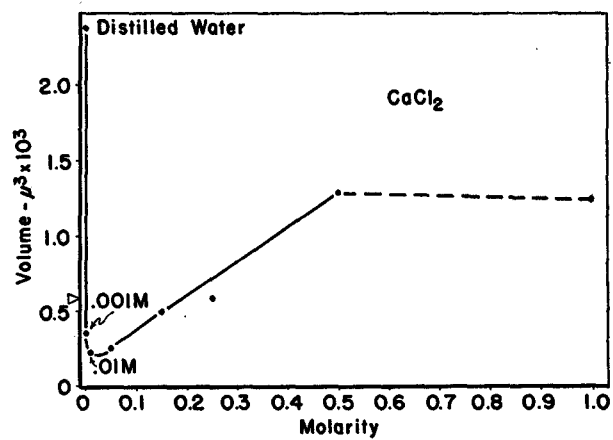
TEXT-FIGS. 3 to 8. The effects of various solutions on the volume of rat liver nuclei. Nuclei were isolated in a salt-sucrose buffer solution and then washed in the solutions under investigation. Mean volumes of 11 preparations in the salt-sucrose buffer solution (4) and distilled water are added to each graph for reference. Each point on the curves represents the mean volume of 46 to 56 nuclei from 4 rats. Values for points connected by broken lines in MgCl_2 and CaCl_2 graphs are uncertain since marked nuclear disintegration occurs in these concentrations and the intact nuclei measured may not constitute a random sample.



TEXT-FIG. 6



TEXT-FIG. 7



TEXT-FIG. 8

narrow range of volumes (580 to 800 μ^3) with the highest peak frequency at about 650 μ^3 . A small group had a peak at 350 μ^3 , thus giving a volume ratio between the two peak frequencies of 1:1.9. A few nuclei were considerably larger but did not constitute a well defined group. The picture was essentially the same for each rat. The distribution was similar to that found for nuclei of fixed tissue (22, 23, *cf.* 24). However, fixed nuclei are considerably smaller, the peak value for the main group being 248 μ^3 as given by Rather (23) as compared with 650 μ^3 for our unfixed nuclei.¹

The similarity of distribution for fixed nuclei and those prepared by centrifugation indicates that the differential centrifugation does not preferentially eliminate one size group. Moreover, the greater proportion of the nuclei used in the volume studies fall within a relatively narrow size range.

Effect of pH

The effect of pH on nuclear volume was studied in a series of solutions containing potassium phosphate buffer salts (0.023 M) and sucrose (0.145 M) in concentrations similar to those used in solution I in the isolation procedure. The results are shown in Text-fig. 3. The volume remained relatively constant between pH 8.91 and 5.12 and decreased at pH 4.64. Extreme shrinkage was observed in very acid solutions (0.1 N HCl). Dilute alkali solutions caused marked swelling and dissolution of the isolated nuclei. Changes in appearance over the pH range 8.91 to 4.64 are described later.

Effects of Electrolytes

Text-figs. 4, 5, 7, and 8 summarize the effects of concentration on volume for the chlorides of Na, K, Mg, and Ca. Two reference points have been placed on each graph: distilled water and solution I (\triangleright on ordinate) which has approximately 0.7 times the isosmotic value of rat blood. These two points are average values taken from 11 preparations and represent 118 and 159 nuclei respectively. Plots of size distribution in experimental solutions (*cf.* Text-figs. 1 and 2) indicate that the volume changes involve the greater proportion of the nuclei and not a single size range. However, with the most dilute and most concentrated solutions in which nuclear disintegration occurs the distribution of sizes is very wide.

The results for NaCl and KCl are nearly identical (Text-figs. 4 and 5). With an increase in concentration from 0.001 M to 0.15 M the volume decreased sharply, exhibiting a change of approximately threefold over this range. The volume was essentially unchanged with further increase in concentration to 1.0 M. The stability of the volume at a concentration of 1.0 M is interesting in view of the fact that this solution extracts nucleohistone (25). Nuclear volumes in potassium phosphate buffer at pH 7.1 (Text-fig. 6) were very similar to those in unbuffered KCl solution of the same molar concentration.

¹The reported values for the highest peak frequency differ somewhat.

Quantitatively, divalent and monovalent cations are markedly different in their effects on nuclear volume, though the general trends are the same for both (Text-figs. 4, 5, 7, and 8). The similarity would be much less apparent had only the range from one-half to twice the isosmotic values been considered, for here with an increase in concentration the volume in the monovalent salts would have been found to decrease slightly and then level, whereas the divalent salts would have given a marked increase in volume. Shrinkage was more marked for the divalent cations at each concentration on the left arm of the curve and the minimum volume was about one-half that of the monovalent cations. CaCl_2 gave more marked shrinkage than MgCl_2 at the two lowest, and greater swelling at the two highest concentrations. The minimum volume occurred in 0.01 M CaCl_2 and 0.05 M MgCl_2 . In none of the electrolyte solutions was there any evidence of wrinkling of the nuclear envelope although

TABLE I

The Effect of Different Salt-Sucrose Mixtures on the Average Volume of Isolated Nuclei

The salt solution contained 0.0094 M KH_2PO_4 , 0.0125 M K_2HPO_4 , 0.00151 M NaHCO_3 , and is that used in the isolation medium minus sucrose. pH 7.1. Nuclei in this series were photographed with the light field microscope. The volume shown for salt-free sucrose indicates only extreme swelling and not a precise final size since many nuclei ruptured in this solution.

No. of nuclei measured	Solution	Mean Volume
		μ^3
35	Salts—no sucrose	618
20	Salts—0.11 M sucrose	697
134	Salts—0.145 M sucrose	671
33	Salts—0.44 M sucrose	597
7	0.88 M sucrose, no salts	3,395

at the minimum volumes some of the nuclei exhibited an angular outline. Surface deformations in the form of optically empty blebs were observed, however, and will be discussed in a later paper. At 0.5 M and 1.0 M CaCl_2 and MgCl_2 the nuclear envelope underwent gradual rupture and fragmentation. Many damaged nuclei were also observed in 0.25 M solutions of these salts and the nuclear volumes shown in Text-figs. 7 and 8 for concentrations above 0.15 M should therefore be taken as indicative of a general trend only.

It is clear that isolated rat liver nuclei do not exhibit typical osmotic behavior in the electrolyte solutions thus far considered.

Effect of Sucrose

Sucrose was of interest both because of its molecular size and its wide use in isolation media. It was found to be without appreciable osmotic effect on the isolated rat liver nucleus. Even in markedly hyperosmotic solutions (0.88

m) nuclear swelling comparable to that in distilled water (Fig. 5) occurred. Further, sucrose had little effect on nuclear volume when added to a salt mixture (Table I). A comparable series of observations on rat kidney nuclei gave similar results.

With swelling in sucrose the optical density was such that the nuclei became very difficult to see either with light field or phase contrast illumination. After several minutes many of the nuclei were observed to rupture with consequent loss of nuclear contents leaving a nuclear "ghost." The swelling and hyaline appearance of nuclei in sucrose have also been described by Ris and Mirsky (14).

Treatment with pure sucrose solutions appears to have been successfully used without undue nuclear damage in several techniques including the preparation of a "nuclear fraction" (26) and the preparation of nuclei for the methyl green method for the histochemical determination of highly polymerized DNA (27). The success of these procedures probably depends on the presence of traces of salts as is suggested by the following observation. If a pure sucrose solution is made to flow continuously past nuclei under the coverslip, swelling and eventual disintegration occur. If the flow is stopped before extensive damage occurs, the nuclei will slowly shrink presumably due to leaching of salts from the glass.

Effects of Electrolytes on the Interior of the Nucleus

The evidence presented thus far indicates that volume changes in electrolyte solutions are brought about through action on the nuclear substance rather than through osmotic effects. The action on the interior is further emphasized by optical changes produced by ions on the nucleus as summarized below. The reference condition has been the appearance of nuclei with phase contrast illumination in uninjured rat liver cells in Krebs' bicarbonate solution. Here the nuclei are perfectly spherical, free of granulation so far as can be determined, and with the nucleoli faintly visible. Nuclei in solution I have a similar appearance (Text-fig. 9). The summary below indicates the chief differences from the reference condition and supplements the data on volume changes previously described (Text-figs. 3 to 8).

Potassium Chloride.—

- 1.0 M. Nuclei less dense under phase contrast. Membrane prominent. Many small granules. Nucleoli shrunken.
- 0.15–0.5 M. Apparently slight reticulation in 0.5 M. Similar appearance but slightly more granulation in 0.25 M and 0.15 M. Nucleoli increase in size with decrease in concentration.
- 0.05 M. Evidence of very slight granulation.
- 0.01 M. Similar to salt-sucrose mixture but larger.

Sodium Chloride.—Similar to KCl with possible coarser granulation in NaCl. (See also reference 14.)

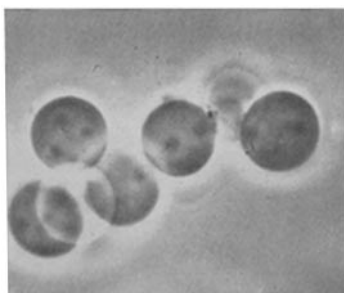
Calcium Chloride.—

1.0 M and 0.5 M. Marked decrease in density under phase. Many nuclei ruptured. Many small granules; no reticulation. Nucleoli extremely shrunken and irregular.

0.25 M. More dense than 0.5 M. Granules as in 0.5 M with additional fine granulation throughout (Fig. 1; cf. Fig. 2).

0.05 M; 0.01 M; and 0.001 M. Very dense and “brilliant” under phase, many irregular in shape. Dark, condensed, irregularly shaped nucleoli.

Magnesium Chloride.—Similar to CaCl_2 but effects less marked.



TEXT-FIG. 9. Nuclei in buffered salt-sucrose isolation medium. Nuclei free of granulation; nucleoli visible. Phase contrast; $\times 1130$, 4 mm. objective.

Potassium Phosphate Buffer.—

1.0 M. Granulation and fine reticulation. Nucleoli dense and shrunken.

Similar results in 0.5 M.

0.25–0.05 M. Similar to solution I (Fig. 4).

0.01–0.001 M. No granulation. Nucleoli swell with decreasing concentration (Fig. 3).

pH (Potassium Phosphate Buffer).—

pH 6.1–8.9. No apparent change in optical density. No granulation. Nucleoli swollen at pH 8.9 but decrease in size with decreasing pH.

pH 5.5. Very fine granulation.

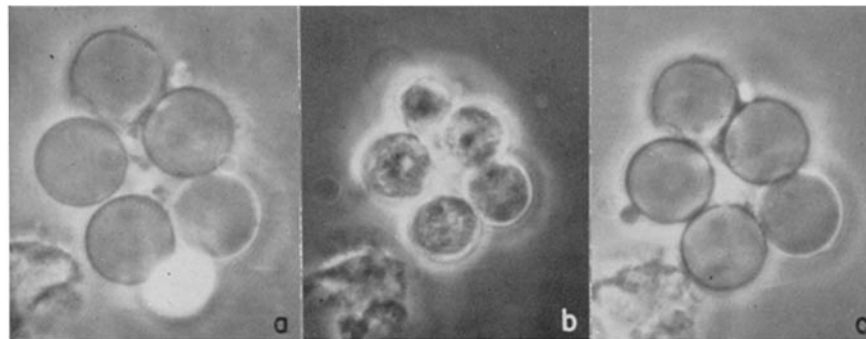
pH 5.1. Fine reticulation. Nucleoli very dense.

pH 4.64. “Brilliant,” shrunken.

Perhaps the most striking effect observed was the marked shrinkage and granulation in CaCl_2 and MgCl_2 , particularly in the former. CaCl_2 was found

to produce its characteristic action in the presence of 0.15 M KCl. Also, 0.88 M sucrose which alone caused marked swelling and eventual disruption did not prevent the shrinkage and granulation in 0.0015 M CaCl_2 . It may be noted that the mixture of 0.88 M sucrose and 0.0018 M CaCl_2 has been used in nuclear isolation (28).

Since both monovalent and divalent cations cause granulation and in view of the marked shrinkage given by CaCl_2 in the presence of monovalent ions it is to be expected that the inorganic salt mixtures of physiological solutions will bring about granulation and shrinkage. This was found to be the case with Locke's solution; Chambers' intracytoplasmic indifferent solution (29) (KCl 0.120 M + NaCl 0.013 M + CaCl_2 0.003 M); Kassel and Kopac's (30)



TEXT-FIG. 10 *a*, *b*, and *c*. Reversibility of changes produced by MgCl_2 in isolated rat liver nuclei. (*a*) 5 nuclei in buffered salt-sucrose isolation medium. (*b*) Same nuclei in 0.01 M MgCl_2 . Note shrinkage and granulation. (*c*) The nuclei have regained their original appearance on return to the original medium. Photographed with oil immersion phase contrast objective which gives less dense image than 4 mm. phase objective used in Text-fig. 9. $\times 1130$.

solution (KCl 0.042 M + NaCl 0.017 M + CaCl_2 0.0015 M + MnCl_2 0.001 M); and with serum itself. The nuclei remain essentially unaltered in citrated plasma, indicating the action of divalent ions in normal serum.

Reversibility

Changes in nuclear volume and optical appearance which occur in the more dilute salt solutions are both readily reversible as previously demonstrated by Ris and Mirsky (14). This can be shown by placing rat liver nuclei which have been isolated in solution I into 0.01 M MgCl_2 solution in which they quickly become granular and shrunken. On returning them to solution I the original appearance is regained at once (Text-fig. 10 *a*, *b*, and *c*). Similarly, nuclei which have become enlarged and optically empty after short exposure to 0.88 M sucrose decrease again in volume and the nucleoli reappear if the

nuclei are returned to solution I. Also the very marked shrinkage occurring in nuclei in 0.1 N HCl is reversed by solution I. Swelling after treatment at such a low pH would not be expected of a protein having an isoelectric point in the acid range. Moreover, histone would be removed by such acid treatment. Nucleic acid would appear to be the substance concerned in these volume changes as has been previously pointed out (14).

Desoxyribonuclease and Volume Changes

In view of the probable involvement of nucleic acid in nuclear volume changes the effect of depolymerization of desoxyribonucleic acid (DNA) was examined. Nuclei were treated with crystalline desoxyribonuclease (DNAase) (Worthington; 1 mg./ml.) in solution I for 2 minutes and the response to experimental solutions followed photographically. The typical swelling in distilled water and shrinkage in 0.001 M CaCl_2 did not occur after treatment with DNAase, though there were minimal changes in these solutions. The enzyme itself caused a slight volume decrease. Nuclei which had undergone a decrease in volume in 0.001 M MgCl_2 and were treated with DNAase while shrunken did not show the customary return to their original volume in solution I. The experiments with DNAase demonstrate that treatment of nuclei with this enzyme prevents both swelling and shrinkage resulting from changes in the ionic composition of the medium.

DISCUSSION

The organization of compounds within the living nucleus of mammalian cells is not well known, though concepts of the general state of the nucleus have not been wanting. Thus it has been considered to have the properties of a sol without internal structure (31); a reticulum with a sol-like liquid as the continuous phase (32); a thixotropic gel (33); a coacervate (10, 12); and a structure completely filled by the individual chromosomes (14). These states are not mutually exclusive as Ris and Mirsky (14) have pointed out; moreover, there is abundant evidence that the state of the nucleus can change under experimental conditions (31, 34). Similarly, the reactions of isolated nuclei will undoubtedly differ depending upon the method of isolation, the media used, and the source of the nuclei. For the present, observations on isolated nuclei should be considered as characterizing the technique and not as necessarily giving information on the intracellular condition.

The present study, though not designed primarily as an investigation of the organizational state of the nucleus, does bring out certain features of the behavior of the nuclear colloid. These are: (1) marked non-osmotic volume changes with change in electrolyte composition and concentration; (2) reversibility of alterations in volume and granulation; and (3) dependence of volume changes on polymerization of DNA.

Churney (19) in discussing the physicochemical properties of animal nuclei considers that the nuclei of eggs of certain marine invertebrates exhibit osmotic behavior but that the deviation from the Boyle-van't Hoff law (volume \times pressure = k , where k is a constant) becomes greater with decreasing concentration of the external medium. In the present studies it is evident (Text-figs. 4 to 8) that the isolated rat liver nucleus in electrolyte solutions does not conform to the Boyle-van't Hoff relationship over any considerable range of concentrations and that even in a limited range of the more dilute solutions the volume changes are relatively small and a very large non-osmotic volume would have to be assumed. The most favorable case is found with KCl solutions. Here the product of volume and pressure is relatively constant for the concentrations 0.25 M, 0.15 M, and 0.05 M, if one assumes a non-osmotic volume of 89 per cent in 0.15 M solution. In view of the changes in volume resulting from alterations in the nuclear colloid discussed below it appears that osmotic behavior, if present at all, plays a secondary role in volume changes of the isolated rat liver nucleus. Callan (20) and Goldstein and Harding (21) found that the amphibian germinal vesicle was permeable to salts but not to albumin. Frog erythrocyte nuclei were also freely permeable to salts injected into the cell (37). Because of the possibility that the nuclear membrane may be altered during isolation we cannot say whether the permeability to salts found *in vitro* also obtains within the liver cell.

Quite apart from possible osmotic volume changes within the cell it appears likely that volume may also reflect the state of the nuclear colloids. The differential sensitivity of nuclear volume to low concentrations of monovalent and divalent cations indicates a colloidal system having a net negative charge. As the cation concentration was increased and the charge neutralized the nuclear volume decreased to a minimum (see Text-figs. 4-8). With further increase in concentration the volume remained constant with monovalent cations but with divalent cations the volume increased again, presumably as a result of reversal of charge. The calcium ion was more effective than the magnesium ion in this respect. The negatively charged substance concerned is probably DNA.

Direct evidence that the capacity of the nucleus to show volume changes depends upon polymerized DNA is shown by the inhibitory action of DNAase in preventing both swelling and shrinkage. Not only will the capacity for size changes depend upon DNA but the size and appearance of the nucleus will be a function of the molecular arrangement of the DNA as influenced by electrolyte environment. This interpretation is similar to that of Ris and Mirsky (14) who consider that the chromosomes in the extended state completely fill the nucleus and that swelling and shrinkage of the nucleus are the result of volume changes in the chromosomes which will in turn be governed by the state of the highly polymerized DNA of the chromosomes. If

the chromosomes in the extended state determine the nuclear volume, then we might expect that with a constancy of chromosome number and composition any differences in nuclear volume, in different tissues for example, would reflect cytoplasmic differences in electrolytes, etc. Further, nuclei with the same chromosome composition which may differ in volume in different tissues should assume the same volume when isolated in the same medium, unless the effects of the cytoplasm are not reversible. Studies concerned with these aspects are needed.

From the effects of electrolytes on isolated nuclei it may be suggested that no appreciable amounts of *free* divalent cations are present in the nucleus normally since the characteristic granulation produced by these ions is absent in the uninjured cell. On a similar basis one could look for possible explanations of the changes which occur when a nucleus is transferred from one ameba to another of a different species (35) or on mechanical injury (36) in alterations of the ionic composition of the cytoplasm.

In the same way that optical properties of the nucleus may be used as criteria of normalcy or injury they may be employed in defining the adequacy of a medium. From the optical appearance it has been possible to say that certain electrolyte solutions, particularly those containing divalent cations, bring about an alteration from the state in the uninjured cell. These include Locke's solution and serum itself. In view of the difference in composition between extracellular fluids and cytoplasm we should scarcely expect a medium satisfactory for a cell to be adequate also for its isolated components. From the results on the electrolyte solutions thus far examined it may be suggested that potassium phosphate approximately 0.02 to 0.05 M with a pH near neutrality is a suitable first approximation for a nuclear medium. The changes in appearance with small differences in concentration and pH are not sufficiently distinct to permit us to set more precise values. In addition to the potassium phosphate buffer the isolation medium employed here contains bicarbonate, since this is present in rat liver, and sucrose to provide suitable density for differential centrifugation. As more information becomes available on changes in nuclei during isolation, it should be possible to devise additional criteria for the evaluation of more complex nuclear isolation media.

We are grateful to Miss Betty J. Martin for her competent assistance throughout this study.

SUMMARY

1. The effects of inorganic ions, electrolyte concentration, and pH on the appearance and volume of the isolated rat liver nucleus have been studied. Nuclei were isolated by differential centrifugation in a buffered salt-sucrose mixture at pH 7.1. Nuclear volumes were determined photographically.

2. In solutions of NaCl, of KCl, and in potassium phosphate buffers the

nuclear volume decreased markedly with an increase in concentration from 0.001 M to 0.05 M but remained essentially constant with further increase in concentration to 1.0 M. The effects of CaCl_2 and MgCl_2 differed from those of NaCl and KCl in that a smaller volume was obtained in concentrations less than 0.15 M, and in the case of CaCl_2 an increase in volume was obtained in more concentrated solutions. The volume changes are considered to be due primarily to ionic effects on the nuclear colloids rather than to osmotic behavior.

3. Treatment of nuclei with DNAase prevented the characteristic volume changes resulting from ion effects, suggesting the importance of DNA in nuclear volume changes.

4. The optical changes in isolated nuclei in various concentrations of KCl, NaCl, CaCl_2 , MgCl_2 , and in potassium phosphate buffers as observed under phase contrast illumination are described. CaCl_2 gave the most marked nuclear changes from the conditions in the uninjured cell and caused shrinkage and granulation in 0.001 M concentration. The effects of CaCl_2 were also manifested in 0.88 M sucrose, in mixtures with monovalent salts, and in serum. Changes in nuclear volume and optical appearance which occurred in salt solutions and in 0.1 N HCl were readily reversible.

5. Nuclear volume remained constant between pH 8.91 and 5.12 and decreased in more acid solutions.

6. Sucrose had no appreciable osmotic effect, and in hyperosmotic solution (0.88 M) nuclei showed swelling and rupture comparable to that in distilled water.

7. The results are considered in relation to the requirements of nuclear isolation media.

8. Rat liver nuclei isolated in a buffered salt-sucrose medium by differential centrifugation exhibited a pattern of size distribution similar to that of fixed nuclei but were of considerably larger volume. The ratio of the volumes of the peak frequencies of the two chief size groups was 1:1.9.

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EXPLANATION OF PLATE 2

Rat liver nuclei. Phase contrast. Magnification 1065.

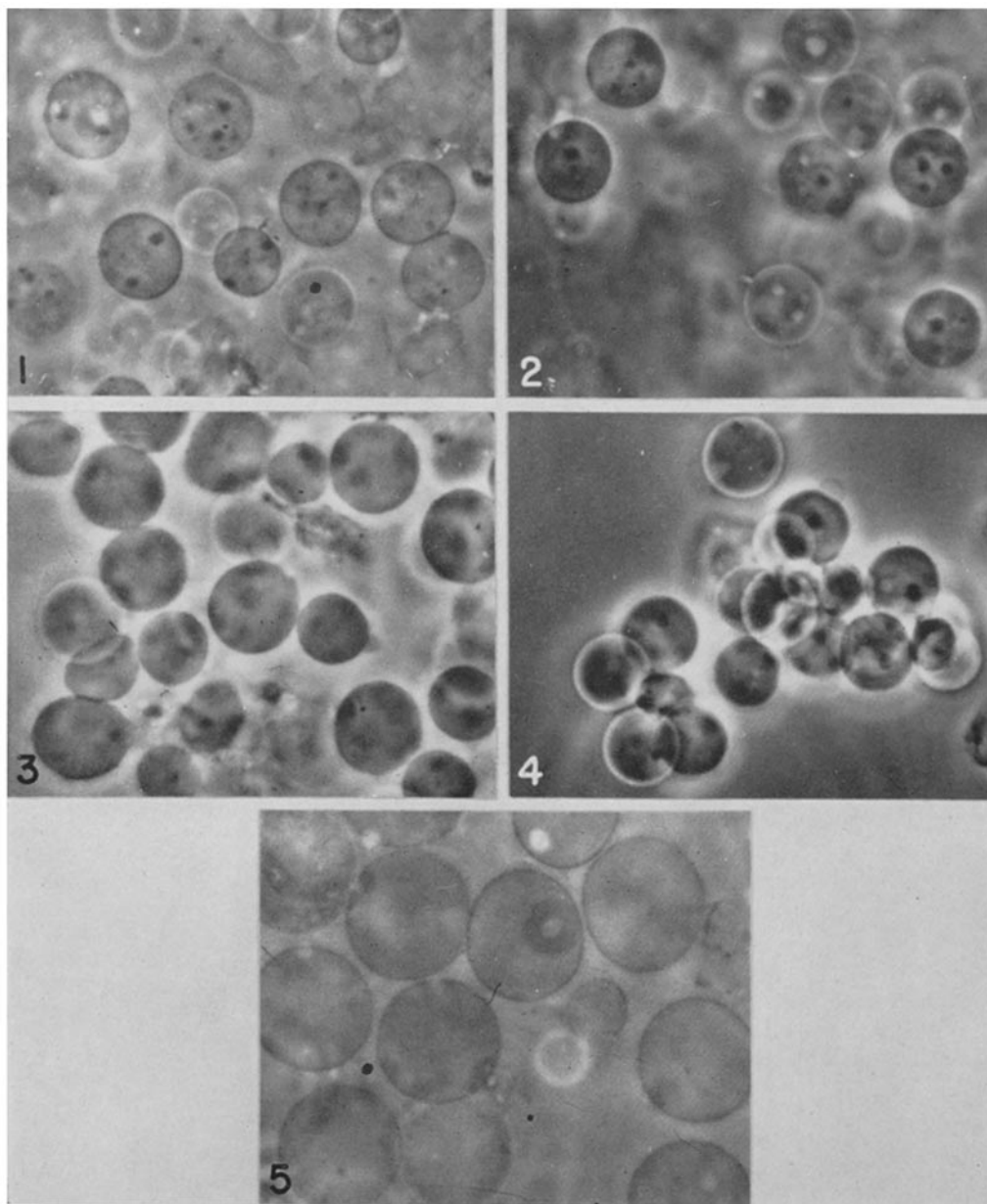
FIG. 1. CaCl_2 , 0.25 M. Granulation marked. Nuclear remnants indistinctly shown. Nucleoli small and dense.

FIG. 2. CaCl_2 , 0.15 M. Less granulation than in 0.25 M.

FIG. 3. $\text{K}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$, 0.01 M; pH 7.1. No granulation. Nucleoli indistinct. Nuclear volume greater than in 0.15 M (Fig. 4).

FIG. 4. $\text{K}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$, 0.15 M; pH 7.1. No granulation. Bleb on nucleus just above center.

FIG. 5. Distilled water. Note extreme swelling. Nuclei almost invisible when observed directly.



(Anderson and Wilbur: Studies on isolated cell components. IV)