STUDIES ON ISOLATED CELL COMPONENTS

V. THE EFFECTS OF VARIOUS SOLUTIONS ON THE NUCLEAR ENVELOPE OF THE ISOLATED RAT LIVER NUCLEUS

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THE existence of a distinct nuclear membrane is no longer in serious doubt (10, 18, 21, 13, 15, 8). A number of observations have suggested that this structure is composed of at least two layers. Thus the nuclear envelopes of several amphibian oöcytes have been shown to consist of a perforated inner sheet or layer and a thin, apparently structureless outer layer (8). In isolated plant nuclei, blisters or blebs have been observed to rise from the main body of the nucleus (11, 14), while similar observations have been recorded for rat liver nuclei (3). Blebs have also been observed in nuclei of cells after treatment with neutral formalin (12). From birefringence studies it has been concluded that the envelope generally contains protein chains or plates running in a tangential direction (19) and also a variable amount of lipid (20). From similar studies Monné has concluded that the nuclear envelope is double with a firm nuclear protein layer free of lipids, and a very delicate cytoplasmic protein-lipid layer (18). Baud has also presented evidence for a perinuclear lipidic layer in the liver cell (6) based on histochemical techniques. These results, as well as those obtained by microdissection methods (9), demonstrate that the nuclear envelope is a structural entity and not merely a phase boundary. In view of the complexity of this structure and its evident lack of many of the properties which have come to be associated with the cell membrane (10, 16, 17, 7), it has been considered advisable in the present work to use the term "nuclear envelope" in place of nuclear membrane.

The nuclear envelope has been shown to be permeable to salts (1, 5, 8, 16, 17, 7) and to many low molecular weight substances (7). It appears to offer little resistance to the entrance of heparin or to the extrusion of

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highly polymerized desoxyribonucleic acid (DNA) (4). A number of proteins have been shown to penetrate it rather readily (2),

In the present study the results of observations of the effects of various solutions on the envelope of isolated rat liver nuclei will be presented. A number of factors influencing the formation of nuclear blebs or blisters have been investigated. As will be shown, the isolated nucleus possesses an envelope with properties not dissimilar from those previously described for nuclei of intact cells. The results obtained are discussed from the dual point of view of their possible application to nuclear physiology and to the evaluation of techniques for the isolation of nuclei.

EXPERIMENTAL

Methods. Rat liver nuclei were isolated in a phosphate-bicarbonate buffer containing sucrose[1] as previously described (22). Observations and photographs were made at room temperature (24–31° C) with the phase contrast microscope (5). All solutions were made up in double distilled water.

Observations on the effects of salt solutions. In a number of solutions, blebs or bubbles have been observed to appear on the surface of isolated nuclei. The blebs are generally rather small except in dilute solutions of $CaCl_2$ and $MgCl_2$ where blebs were often as large as the nuclei. Blebs which occur in 0.001 M $MgCl_2$ are shown in Fig. 1. It should be noted that blebs in contact with each other do not coalesce in a manner characteristic of soap bubbles, but remain discrete.

The effect of a graded series of concentrations of KCl, NaCl, K-phosphate (pH 7.0–7.4), $MgCl_2$, and $CaCl_2$ on the formation of blebs by isolated rat liver nuclei is shown in Table I. In many of the solutions the blebs were numerous but small (ca. 1–3 μ). This was especially true of dilute NaCl, KCl, K-phosphate solutions, and also distilled water. It should be noted that the figures given in Table I are minimal, since only blebs which could be seen on photographs were counted. No blebs have been observed in the isolation solutions (phosphate-bicarbonate-sucrose) in any of the numerous preparations made. Blebs were observed to occur most frequently in 0.001 M KCl. Almost as many were seen in 0.001 M $MgCl_2$. In the latter instance the blebs were generally very large (Fig. 1) and in some instances

[1] The following solutions have been used for isolating nuclei in the present series of studies (22): *Solution I.* 0.0094 M KH_2PO_4, 0.0125 M K_2HPO_4, 0.0015 M $NaHCO_4$, 0.145 M sucrose. Solution II. Same salt concentration as Solution I, sucrose 0.218 M. *Solution III.* Same salt concentration as Solution I, sucrose 0.272 M.

TABLE I

Bleb Formation in Various Salt Solutions.
Results are expressed as number of blebs observed per 100 nuclei.

Solution	Molarity						
	1.0	0.5	0.25	0.15	0.05	0.01	0.001
NaCl	44	51	48	62	63	33	45
KCl	48	36	30	38	34	75	86
$CaCl_2$	0	0	13	7	46	69	73
$MgCl_2$	0	0	4	9	23	20	79
K-Phosphate (pH 7.02–7.37)	6	6	40	42	64	39	75

Double distilled water gave an average of 66 blebs per 100 nuclei, while none was observed in 3 times isotonic sucrose (0.88 M).

appeared to equal the nucleus in volume. A thicker component of the nuclear envelope appears to be invaginated beneath the blebs. When a preparation was observed for 20–30 minutes, many of the blebs could be seen to leave the nuclei as structureless spheres floating free in solution.

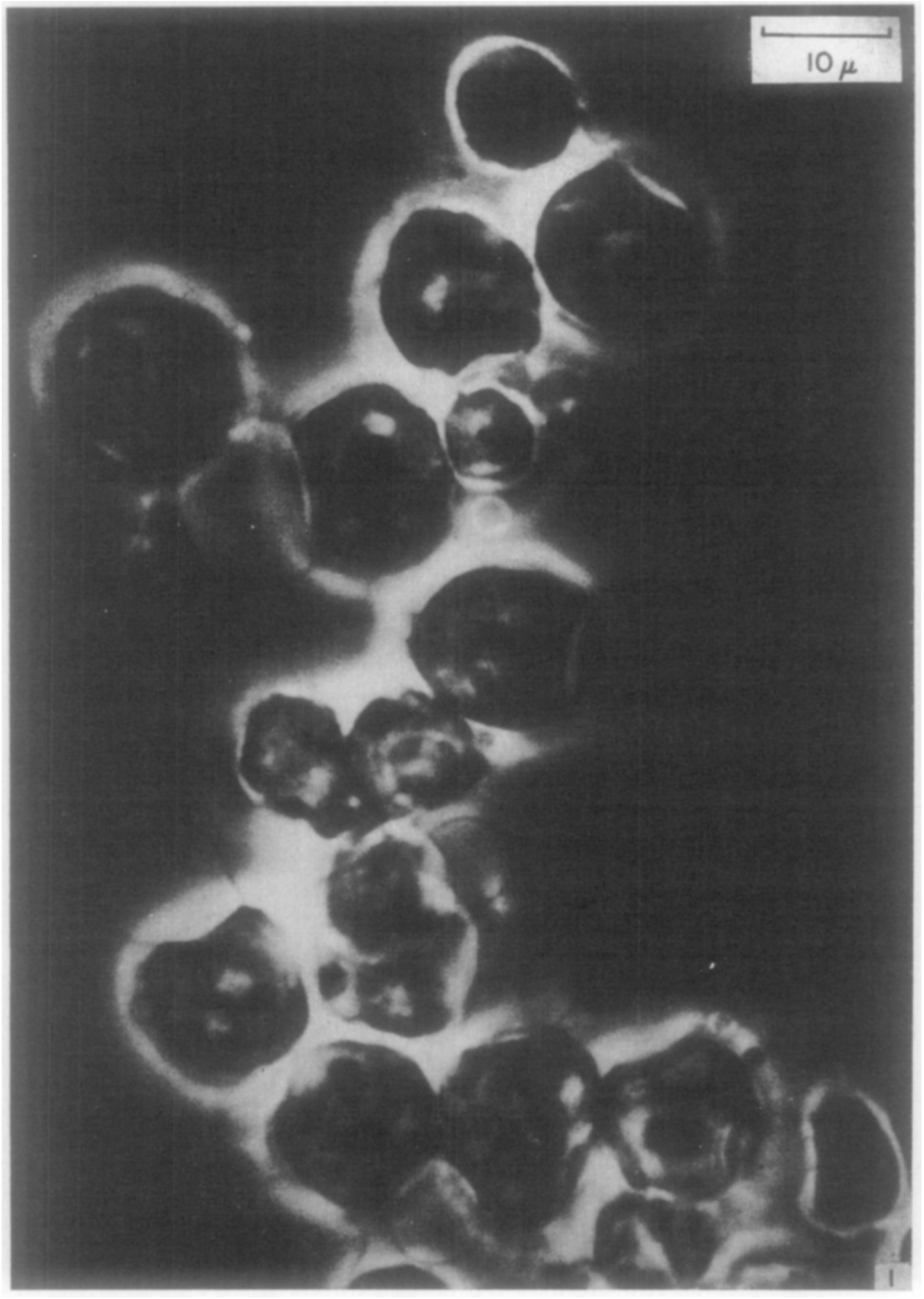
The formation of blebs was found to be a readily reversible phenomenon. Blebs formed by treating nuclei with 0.001 M $MgCl_2$ and which had not detached themselves from the nuclei, rapidly returned to the nuclear surface when the nuclei were replaced in Solution I (salt-sucrose isolation medium) (Figs. 2, 3). The reversibility of changes in nuclear volume and appearance in these solutions has been discussed in a previous publication (5).

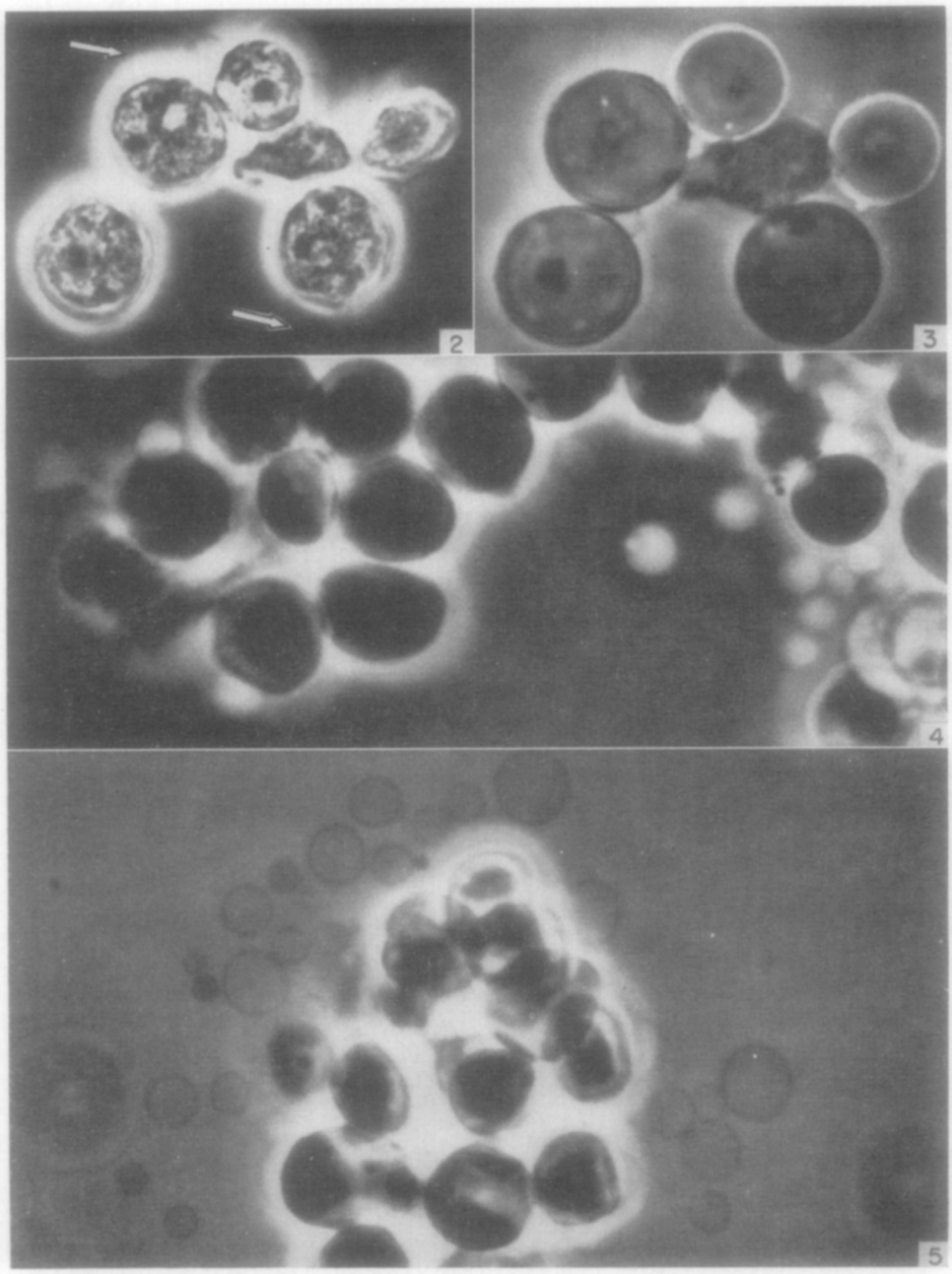
Observation has revealed blebs forming on nuclei inside the whole cells found in crude homogenates. However, it is not known whether these cells were uninjured.

The effect of sucrose on bleb formation. Bleb formation was observed when sucrose was omitted from the isolation medium (Solution I). No other change in either diameter, shape, or internal structure was seen. Reintroduction of the complete medium caused the blebs to disappear. The complete absence of bleb formation in the isolation medium is therefore due to the presence of sucrose.

The effect of sucrose in a number of concentrations (0.10, 0.05, 0.01,

Fig. 1. Isolated rat liver nuclei in 0.001 M $MgCl_2$. Note formation of numerous noncoalescing blebs and invagination of the thicker nuclear envelope component under the blebs. (American Optical Company dark medium phase contrast plates.)


10 μ


2
3
4
5

and 0.001 M) in 0.001 M $MgCl_2$ was studied to determine the range of concentrations over which sucrose prevented bleb formation, which was first evident in 0.01 M sucrose+0.001 M $MgCl_2$. Blebs in the latter solution were very small, however, and did not exceed 2 or 3 μ in diameter. In 0.001 M sucrose+0.001 M $MgCl_2$ large blebs were observed. It should be noted that sucrose appears to partially counteract the shrinking effect of very low concentrations of $MgCl_2$ on nuclei.

Observations on the permeability of nuclear blebs. The concentration of solutes inside the nuclear blebs appears to be very nearly the same as that in the various solutions used to produce bleb formation (distilled water, 0.001 M $MgCl_2$, 0.01 M KCl). This conclusion follows from the fact that the image produced in the phase contrast microscope is due to differences in index of refraction and, therefore, may be expected to reflect differences in physical density or solute concentration in the objects observed. If the nuclear blebs contained appreciable amounts of protein, for example, and were surrounded by a protein-free solution, they would appear dark against a gray background when viewed with dark-contrast phase objectives. Blebs impermeable to protein in a protein solution, on the other hand, should appear as bright structures against a gray background. The blebs described are difficult to observe directly, and when photographed with extremely high contrast photographic materials (Fig. 1) show neither appreciable density nor brilliance.

The addition of small amounts of substances which do not appear to penetrate the nuclear blebs results in a marked change in their appearance under the phase contrast microscope. As shown in Fig. 4, the blebs appear as light structures against a dark background when the nuclei are placed in a solution containing 5 per cent partially degraded gelatin[1] (molecular weight ca. 4 000) in 0.001 M $MgCl_2$. Similar results were obtained with bovine serum albumin. It appears from these observations

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Figs. 2 and 3. Same nuclei in 0.001 M $MgCl_2$ and in salt-sucrose medium (Solution I). Note complete reversibility of bleb formation and return to original homogeneous condition. Damaged nucleus in center shows changes similar to intact nuclei. Bleb-like series of faint concentric rings on edge of small nucleus to extreme right in Fig. 3 are due to small dense particle below the focal plane. (American Optical Company dark medium phase contrast plates.)

Fig. 4. Isolated rat liver nuclei 10 minutes after treatment with 5 per cent partially degraded gelatin in 0.001 M $MgCl_2$, showing formation of bright nuclear blebs. Note blebs which have come free of the nuclei. (Zeiss Winkle phase contrast plates, 1 470 ×.)

Fig. 5. Nuclei treated with 5 per cent partially degraded gelatin in Solution I, and then with 0.001 M $MgCl_2$. Note formation of numerous blebs which appear slightly darker than background. (Zeiss Winkle phase contrast plates, 1 470 ×.)

[1] A special preparation originally devised as a blood substitute.

that the blebs are not readily permeable to proteins. When fresh nuclei pretreated with Solution I plus 5 per cent partially degraded gelatin are placed in 0.001 M $MgCl_2$ plus 5 per cent partially degraded gelatin, blebs are again observed which appear as light objects against a gray background. However, when these blebbed nuclei are placed in 0.001 M $MgCl_2$, the blebs become first almost invisible, and then slightly darker than background as the gelatin in the solution around the nuclei is washed away (Fig. 5). This suggests that some gelatin entered the nuclei before the blebs were formed and later became incorporated in them, causing them to appear as slightly darker structures in a protein-free solution. Since this final density difference is not great, it is evident that only a small amount of protein entered the nuclei during the short period (6 minutes) before bleb formation was initiated.

As shown in Figs. 4 and 5 many blebs break away from the nuclei. Unfortunately the osmotic properties of these free blebs could not be studied, because introduction of different solutions under the cover slip washes them away.

All attempts to fix or preserve blebs for study with the electron microscope have so far been unsuccessful.[1] The introduction of fixatives or fat solvents causes the blebs to disappear. These observations, together with the evidence cited previously for a perinuclear lipid layer, suggest that the blebs may be composed chiefly of lipid material.

DISCUSSION

Blebs or blisters have been shown to occur on the surfaces of isolated rat liver nuclei in a variety of salt solutions and in distilled water. More were observed in dilute salt solutions, small ones being seen in very dilute solutions of salts of monovalent cations, while large ones were noted in very dilute solutions of salts of divalent cations. None have been observed in the phosphate-bicarbonate-sucrose solutions used for nuclear isolation in the present studies.

As has been previously noted, the envelope of the isolated rat liver nucleus appears to be permeable to a number of substances of relatively high molecular weight (heparin, highly polymerized DNA, and several proteins). It is unlikely, therefore, that the blebs are formed from a preexisting continuous surface sheet in response to changes in osmotic pres-

[1] Betty Martin, unpublished data — 1951.

sure. Rather, it is thought that the bleb-forming material (presumably phospholipid) is usually dispersed in the nuclear envelope. If the nuclear envelope is composed chiefly of a meshwork of protein fibrils or ribbons tangentially oriented for the most part, the bleb-forming material may be thought either to cover the fibrils or to be intimately associated with them. Such an envelope would allow free passage of high molecular weight substances. Under certain conditions (such as absence of sucrose and very low salt concentration), the bleb-forming material is thought to move to the surface, forming a continuous surface layer which may ultimately rise off to form blebs or blisters. As more of this material moves to the surface, the blebs are able to form complete spheres that break away.

The blebs do not appear to be readily permeable to protein, since blebs in 5 per cent solutions of bovine serum albumin or partially degraded gelatin show marked brilliance in the phase contrast microscope. Since the optical image under these circumstances reflects differences in refractive index (and therefore solute concentration), the fact that the blebs appear as luminous spheres against a dark background indicates that they have a lower refractive index than the surrounding medium, and therefore a lower concentration of protein. Blebs formed by nuclei previously treated with partially degraded gelatin appeared darker than the surrounding medium when placed in a protein-free solution, suggesting that in this instance some protein was trapped inside the blebs and could not readily escape.

It appears, therefore, that the blebs are less permeable than the envelope from which they are formed, and in contrast to the intact nuclear envelope, may behave osmotically. The mechanism of bleb formation, however, is not thought to be due to changes in osmotic pressure *per se*, since blebs have been observed to occur in a wide range of salt concentrations, in 5 per cent solutions of bovine serum albumin and partially degraded gelatin, and in distilled water. Sucrose (0.05 M) has been shown to prevent the appearance of blebs when added to a dilute $MgCl_2$ solution which otherwise caused extensive bleb formation. The inhibiting effect of sucrose does not appear to be due, necessarily, to its osmotic pressure. Rather, sucrose appears to have a rather specific effect in maintaining the dispersed state of the bleb-forming material. This conclusion is supported by 1) the observation that 0.145 M sucrose in 0.001 M $MgCl_2$ does not cause the blebs to shrink to a smaller size but rather causes a complete return to the nuclear surface, and 2) by the finding that bleb formation is prevented by a concentration of sucrose having approximately 1/5 the osmotic pressure of rat blood.

It is of interest to note that the mechanism proposed to explain bleb formation could also serve to produce cyclical changes in nuclear permeability.

Techniques for isolating rat liver nuclei in a condition morphologically similar to intracellular living nuclei still require the use of sucrose. It is not unlikely that nuclei isolated in salt solutions without sucrose have lost at least part of the bleb-forming constituents of the nuclear envelope.

SUMMARY

A number of salt solutions have been shown to cause tenuous blebs or blisters to rise from the surface of isolated rat liver nuclei. These have included solutions of NaCl, KCl, and K-phosphate (pH 7.0–7.4) in concentrations ranging from 0.001 M to 1.0 M, and $CaCl_2$ and $MgCl_2$ in concentrations ranging from 0.001 to 0.25 M. A large number of very small blebs were also observed in distilled water. The largest blebs were observed in dilute (0.001 to 0.01 M) solutions of $MgCl_2$ and $CaCl_2$.

The addition of sucrose in a concentration of 0.145 M was found to inhibit effectively bleb formation in a number of salt solutions, and to cause bleb retraction when added to bleb-forming solutions. Sucrose, in a concentration as low as 0.05 M, has been shown to prevent bleb formation by 0.001 M $MgCl_2$.

On the basis of index of refraction differences observed with the phase contrast microscope, it is concluded that the nuclear blebs are relatively impermeable to protein, but may contain protein which has previously penetrated the nuclear envelope.

A possible mechanism of bleb formation is presented.

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