

## STRUCTURAL CHANGES IN NUCLEAR ENVELOPES DURING ELONGATION OF HEART MUSCLE CELLS

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### INTRODUCTION

It has recently been shown (1) that myocardial nuclei become longitudinally compressed during systole and extended during diastole. Myocardial nuclear length varies directly with the sarcomere length, and there is no slippage of sarcomere segments past the nuclear envelope. At sarcomere lengths corresponding to diastole the nuclear envelope is straight, but for every incremental decrease in sarcomere length a corresponding degree of envelope redundancy<sup>1</sup> is observed. Thus the nuclear envelope shows little elasticity at physiologic sarcomere lengths and is pulled taut at diastolic sarcomere lengths.

In this communication, ultrastructural differences between the nuclear membranes of extended and contracted heart muscle cells are presented. These ultrastructural features may be the basis for functional differences.

### MATERIALS AND METHODS

Male Sprague Dawley rats, between 200 and 250 g in weight, were used. The methods for obtaining hearts in a contracted or hyperdistended state, as well as the protocol for fixation, embedding, and electron microscopy, have been previously described (1). Contracted cells were fixed only with osmium tetroxide, since glutaraldehyde gave poor fixation unless perfused through the coronary arteries, and this latter procedure commonly resulted in ventricular dilatation (with extension of muscle cells) due to aortic valve incompetence at the pressures used. Electron micrographs, at an initial magnification of 15,000, were made of nuclear membranes sectioned perpendicular to the nuclear surface (transverse projection) and in the near-parallel, or tangential, plane ("frontal" projection). 2-3 diameter enlargements of these negatives were used to identify nuclear pores and to confirm the contracted or extended state of the muscle cells. Identifiable nuclear pores were then examined at a negative enlargement of 10 or 13 diameters. Frontal

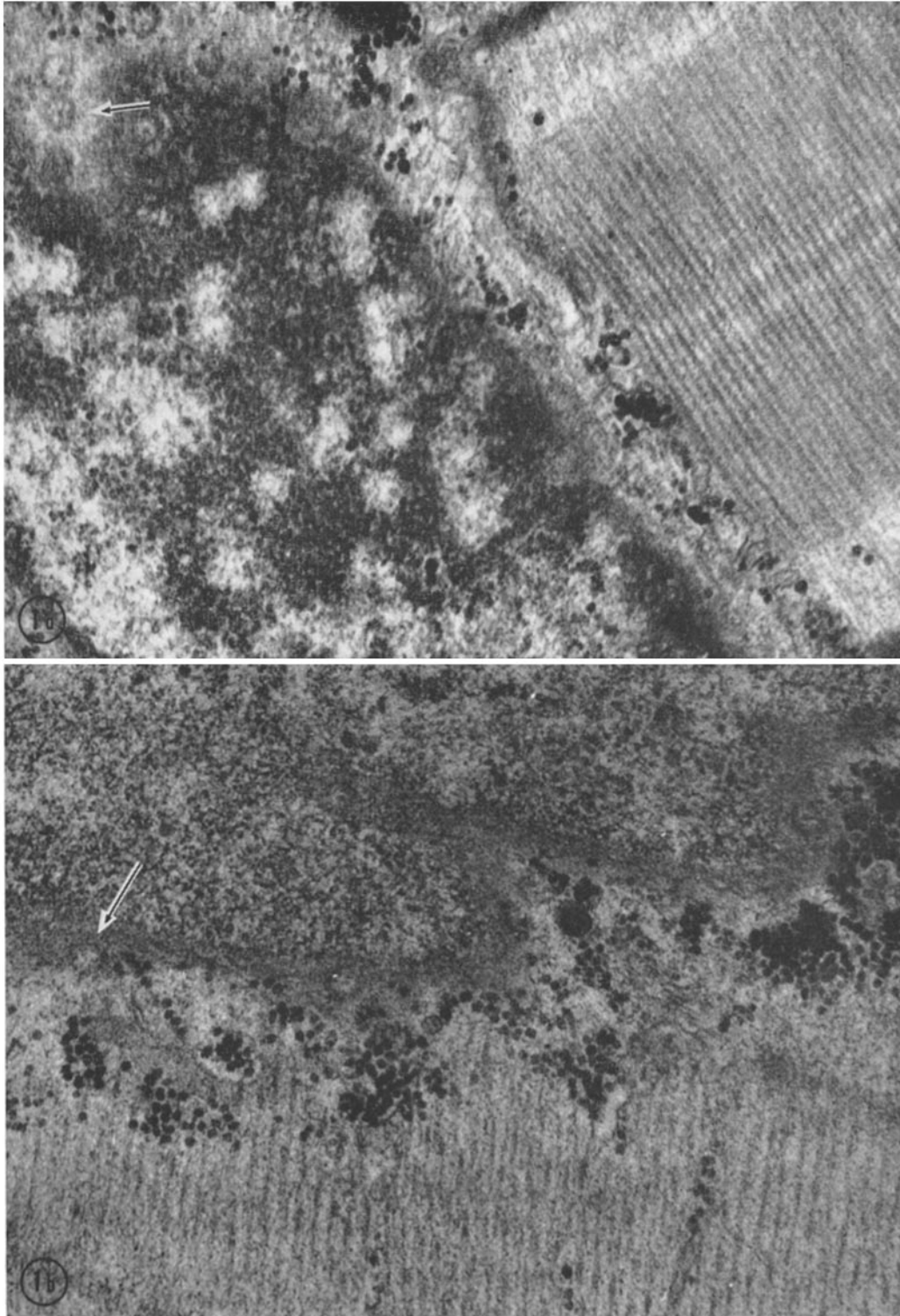
<sup>1</sup> Envelope redundancy is a quantitative expression of folding, and is equal to the per cent increase in envelope length over the straight-line distance between two points (1).

projections were also examined by a modification of the rotation technique of Markham, Frey, and Hills (2). The image of a nuclear pore was projected onto photographic paper after a pin had been placed through the center of the pore image. The paper was then rotated about the pore's axis of symmetry. Multiple exposures were made, and the paper was rotated through a specific arc between each exposure. The criterion for positive identification of a nuclear pore was the presence of a seven-, eight-, or ninefold symmetry demonstrable in a print made with not more than four, five, or six such exposures, respectively. Enhanced images thus obtained were used for measurements of inner and outer annulus diameter, made with a precision caliper. Such images were also used in the search for qualitative differences between the pores of the two populations of nuclei.

### RESULTS AND DISCUSSION

Frontal projections of nuclear pores were easily demonstrable in extended cells but were found only with difficulty in contracted cells (Fig. 1). This was the opposite of what was expected, since the wrinkled nuclear envelopes of contracted cells were more commonly encountered tangentially sectioned than the smooth envelopes of extended nuclei. In transversely cut nuclei, however, pores were demonstrable with equal ease in both groups (Fig. 2). The rotation studies demonstrated the basis of this difference in conspicuousness. As can be seen in Fig. 3, the nuclear pores of extended cells are surrounded by a wide halo which is completely absent around the pores of contracted nuclei. The absence of a distinct pale area around the pores of compressed nuclei renders these more difficult to find.

In the methodology used here, extended nuclei were usually examined in muscle briefly fixed by perfusion with glutaraldehyde and then postfixed in an osmium tetroxide fixation. These methods were adopted because they gave the fewest apparent artifacts. It could be argued, however, that the halo around pores of extended nuclear envelopes is due to the glutaraldehyde fixation, rather than to the *in vivo* structure. Osmium tetroxide fixation might not produce this "artifact," so



**FIGURE 1** Tangential sections of myocardial nuclear membranes showing pores in frontal projection: (a) From a heart distended with glutaraldehyde. Sarcomere length of this cell is  $1.9 \mu$ . Many nuclear pores are seen. They are surrounded by a zone of low electron opacity. (b) From the heart of a rat killed with an intraperitoneal injection of EDTA and KCl producing cardiac arrest in systole (1). Sarcomere length of this cell is  $1.3 \mu$ . The few nuclear pores which are visible are surrounded by electron opaque material. Both  $\times 60,000$ . Arrows indicate pores used for Figs. 3a and 3b.

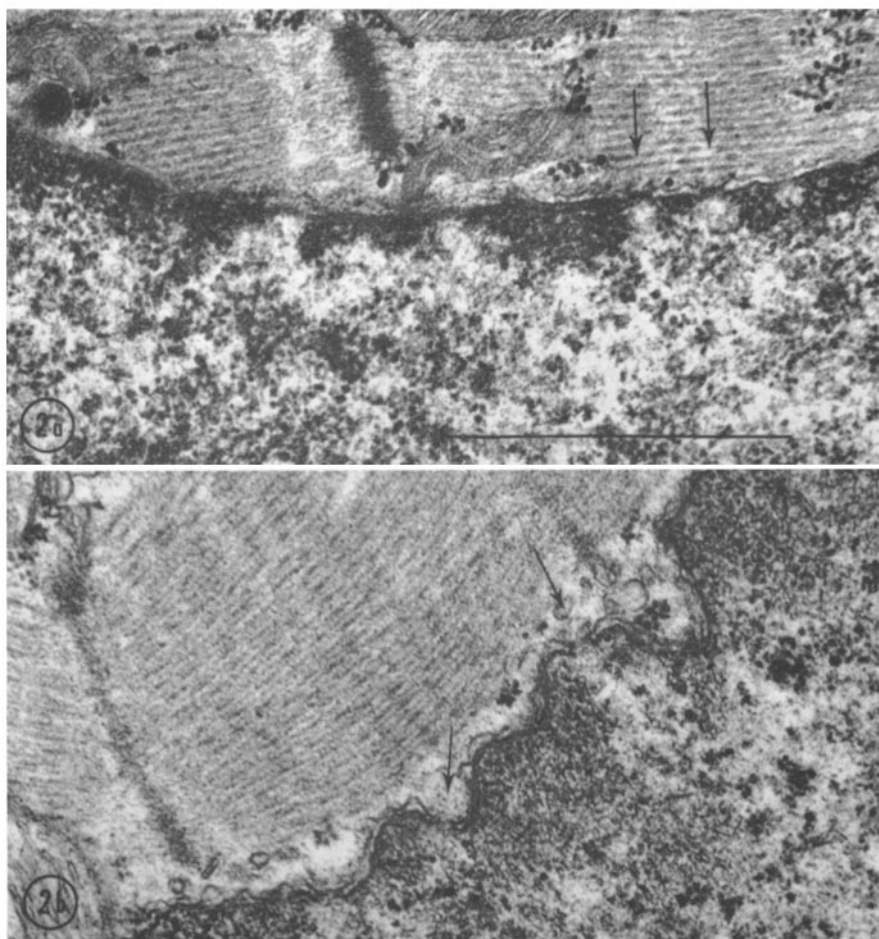
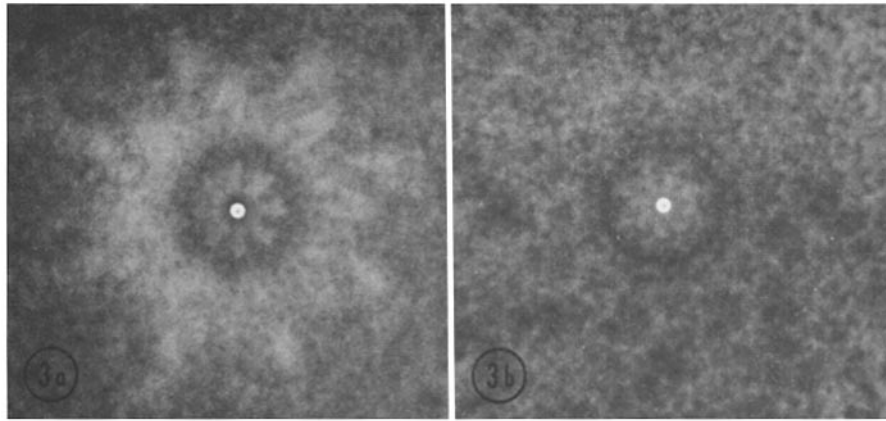


FIGURE 2 Nuclei showing pores (arrows) in transverse projection. (a) From a rat heart distended with glutaraldehyde. Sarcomere length  $2.4 \mu$ . (b) From the heart of a rat killed with an injection of  $\text{CaCl}_2$ . Sarcomere length  $1.4 \mu$ . No consistent difference in appearance or abundance was noted in the nuclear pores examined in this projection. Scale bar in Fig. 2 a is  $1 \mu$ . Both  $\times 45,000$ .

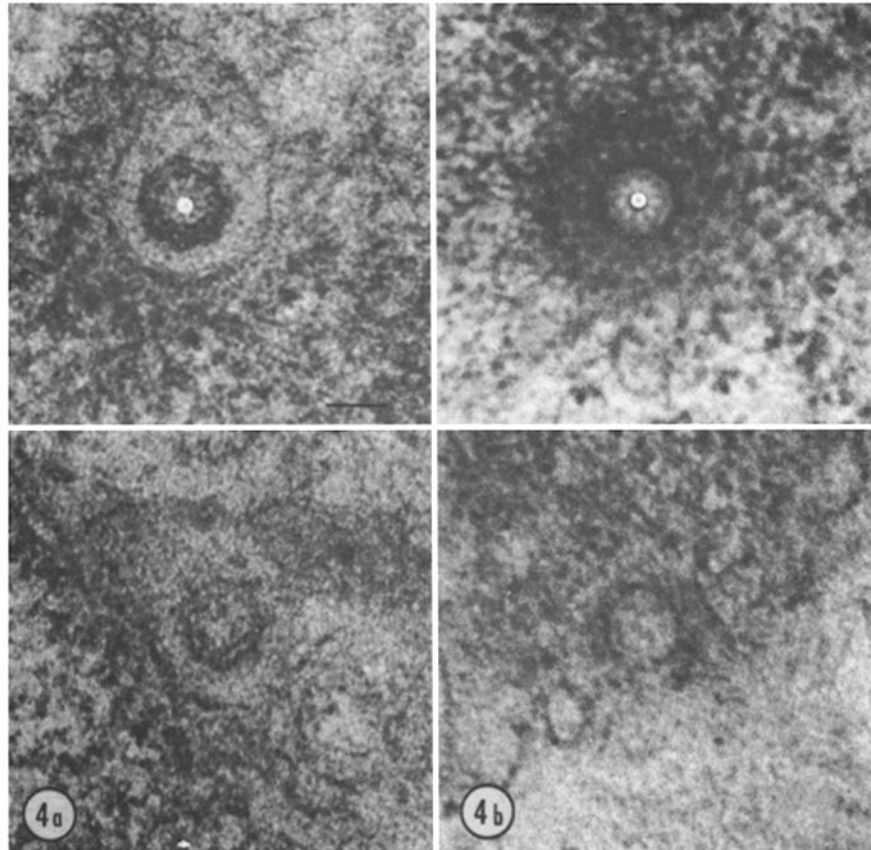
that the difference between the pores of extended and compressed nuclei could be explained completely by postulating different effects of the two fixatives. In order to determine if such fixation artifacts could account for the presence or absence of a periannular halo, the hearts of three rats were fixed by ventricular dilatation and coronary artery perfusion with osmium tetroxide fixative. Almost all muscle cells from these hearts were found to be extremely contracted, and only rare extended cells were found. The longest sarcomere length found was  $1.80 \mu$ , as opposed to a mean sarcomere length of  $2.0 \mu$  for the glutaraldehyde-extended preparations. Because both contracted

and moderately extended cells could be found within a single heart after such osmium tetroxide fixation, it was possible for us to compare pores of nuclei which were extended or compressed, but which came from the same heart. Such pores are shown in Fig. 4. In spite of the fact that maximum sarcomere lengths were not attained, the pores of extended nuclei show a definite halo, while those from compressed nuclei show no halo at all.

Mensuration data from 29 nuclear pores, examined in frontal projection by the rotation technique, are shown in Table I. 13 pores were from extended nuclei and 2 of these 13 were from a heart fixed exclusively with osmium tetroxide.



**FIGURE 3** Frontal projection of nuclear pores examined by the rotation technique. (a) From a rat heart dilated with glutaraldehyde. Sarcomere length  $1.9 \mu$ . Conspicuous halo is present surrounding pore. (b) From the heart of a rat sacrificed with EDTA and KCl. Sarcomere length  $1.3 \mu$ . There is no halo around this pore. Both  $\times 195,000$ ; five exposures separated by an arc corresponding to eightfold symmetry. These pores are shown unrotated in Fig. 1.



**FIGURE 4** Frontal projection of nuclear pores examined by rotation technique. Both pores are from a single heart fixed by distention with osmium tetroxide solution. (a) From cell with a sarcomere length of  $1.80 \mu$ . (b) From a cell with a sarcomere length of  $1.48 \mu$ . A halo is noted around the pore from the cell with the longer sarcomere length, but it is conspicuously absent around the pore from the cell with the short sarcomere length. Four exposures separated by an arc corresponding to eightfold symmetry. Both  $\times 150,000$ . Below each enhanced print is an unrotated ("straight") print of the same pore. Scale bar in Fig. 4 a is  $513 \text{ \AA}$ .

TABLE I  
*Comparison of Nuclear Pores in Compressed and Extended Heart Muscle Nuclei\**

	Contracted	Dilated
Sarcomere length ( $\mu$ )	1.4 $\pm$ 0.009 $\ddagger$	2.0 $\pm$ 0.024 $\ddagger$
Outer annulus diameter (A)	834 $\pm$ 24 $\ddagger$	831 $\pm$ 28 $\ddagger$
Inner annulus diameter (A)	630 $\pm$ 38 $\ddagger$	600 $\pm$ 32 $\ddagger$
Halo		
Present $\S$	1	13
Absent	13	0
Questionable	2	0
Total No. of pores examined	16	13

\* Based on observations of material from 5 rat hearts.

$\ddagger$  Mean  $\pm$  S.E.M.

$\S$  Any clearly delineated pale zone immediately surrounding a nuclear pore, regardless of the size of this zone.

All of these pores showed an easily discernible halo. Of 16 pores from compressed nuclei (from cells with a mean sarcomere length of 1.4  $\mu$ ), 13 had no halo, 2 were questionable, and 1 had a thin halo. Halo width was not measured because the halo perimeter was poorly defined.

Regardless of the method of fixation, or the muscle cell length, there was some variation in the morphology of the nuclear pores. This variation was consistent with descriptions by other workers (3-5), and appears to have no relevance to the compressed or extended state of these nuclei.

#### COMMENT

There is impressive evidence suggesting that nuclear pores play a role in nucleo-cytoplasmic exchange (6-8), and variations in pore morphology can not be used to explain differences in permeability or electrical resistance of nuclei (3). The present study demonstrates a morphological difference in the "nuclear pore complexes" of extended and contracted heart muscle cells. The structures within the annulus have not been described in detail since these do not contribute to a distinction of the two classes of pores studied.

It is not clear what material surrounds the pores as seen in frontal projection, so that we can not explain how this material becomes retracted when the nuclei are stretched. It is possible that the channels through the peripheral chromatin leading to the nuclear pores are the basis of the

observed halo in extended nuclei. If this were the case, it would indicate that these channels change in size as nuclei are compressed and extended, and that their width becomes greater than the outer annulus diameter during extension. However, transverse sections show no apparent difference in the width of the peripheral chromatin channels in the two classes of nuclei studied. Another possibility is that the presence or absence of a halo is due to the configuration of the membrane, such as an area of buckling, with a depression immediately adjacent to the pore. This possibility does not seem likely and was not apparent in our transverse projections. The material studied here thus suggests that buckling of the membrane is not a factor in the development of halos around nuclear pores. However, the data of Gall (4), which demonstrates an accumulation of stain (negative staining technique) around the pore, may be a demonstration of such a depression of the membrane immediately surrounding nuclear pores. It may be worth noting that the nuclear envelopes studied by Gall were stretched prior to fixation.

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