The Nuclear Membranes in Hypertrophied Human Cardiac Muscle Cells

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Nuclear membranes of cardiac muscle cells were studied in 134 patients with cardiac hypertrophy of various causes. Abnormalities observed consisted of: a) increased foldings and convolutions; b) nuclear pseudoinclusions formed by cytoplasmic organelles protruding into saccular invaginations of the nuclear membranes, and c) intranuclear tubules. The increased foldings and convolutions of the nuclear membranes and the nuclear pseudoinclusions appear to result from synthesis of nuclear membranes in excess of that needed to accommodate the increase in nuclear volume which occurs in hypertrophy. Intranuclear tubules were found in 6 patients and consisted of tubular invaginations, 400 to 650 Å in diameter, of the inner nuclear membranes into the nucleoplasm. Some of these tubules were straight and cylindrical, and were associated with a peripheral layer of marginated chromatin; others were not associated with chromatin, appeared coiled and followed irregular courses. Intranuclear tubules in cardiac muscle cells probably represent an extreme cellular response to the stimulus of hypertrophy. (Am J Pathol 78:427–460, 1975)

ALTHOUGH it has long been known that the nuclei of hypertrophied cardiac muscle cells show prominent morphologic abnormalities, including hyperchromasia, enlargement and bizarre modifications of shape, little is known of the ultrastructural aspects of these changes. Indeed, the ultrastructure of nuclei of abnormal cardiac muscle cells has received little attention. During recent studies of hypertrophied cardiac muscle cells in human hearts [1–5] we have observed a spectrum of alterations in the configuration of the nuclear membranes of hypertrophied myocytes. The present communication describes these alterations, which range from increased convolutions of the membranes to the formation of different types of intranuclear tubules, and discusses their significance.

Materials and Methods

The ultrastructural observations reported in this communication were made on cardiac tissues obtained from a total of 134 patients as follows: a) crista supraventricularis muscle resected at operation from 59 patients with various congenital

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cardiac diseases associated with infundibular stenosis [1]; b) ventricular myocardium obtained at operation from the septum and the left and right ventricular free walls from 28 patients with hypertrophic cardiomyopathy (asymmetric septal hypertrophy, ASH)[2,3]; c) left ventricular apical myocardium obtained at operation from 16 patients with aortic valvular disease (6 patients with predominant aortic valvular stenosis, 5 patients with pure aortic regurgitation and 5 patients with combined aortic stenosis and regurgitation)[4]; and d) ventricular myocardium (obtained in 14 patients by Konno catheter biopsy of the right side of the ventricular septum and in 17 patients by Menghini needle biopsy of the left ventricular free wall) from 31 patients with congestive cardiomyopathies of various types.[5]

All tissues were fixed with cold 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, washed with several changes of buffer, postfixed with cold 1% osmium tetroxide in Millonig's phosphate buffer, dehydrated with ethanol and propylene oxide, and embedded in Maraglas.[6] Ultrathin sections were stained with lead citrate and uranyl acetate and examined with the electron microscope.

Results

Observations made on nonhypertrophied human cardiac muscle cells confirmed findings of recent ultrastructural studies which have defined certain aspects of the morphology of nuclei and nuclear membranes of normal cardiac muscle cells in other species.[7-9] The nuclei in nonhypertrophied cells are centrally located and appear as smooth, elongated, oval-shaped structures, the major axes of which are oriented parallel to the myofibrils (Figure 1). The nuclei are limited by the inner and the outer nuclear membranes, both of which are single and trilaminar (Figures 2–4). These two membranes run a parallel course and are separated from each other by a space which measures approximately 200 to 250 Å in width and appears electron lucent. This space hereafter will be referred to as the perinuclear cistern. The outer nuclear membrane is continuous with the membranes which form the tubules of the sarcoplasmic reticulum (Figure 3). Thus, the lumen of the perinuclear cistern is continuous with the lumina of the tubules of sarcoplasmic reticulum. This continuity is evident with respect to tubules of both smooth- and rough-surfaced reticulum (Figures 3 and 4). Occasionally, ribosomes are attached to the outer surface of the outer nuclear membrane. The inner nuclear membrane normally does not form projections or protrusions into the nucleoplasm. The inner surface of the inner nuclear membrane is closely associated with an electron-dense zone of marginated chromatin. Presumably involved in this association are the sites of attachment of the nuclear chromatin to the inner surface of the inner nuclear membrane. A fibrous lamina immediately adjacent to the inner surface of the inner nuclear membrane is prominent in certain cell types,

but it is not present in cardiac muscle cells. The inner and the outer nuclear membrane fuse with each other at points where nuclear pores are formed (Figure 1). Nuclear pores are complex ring-like structures which measure from 700 to 900 Å in diameter (Figures 2, 3 and 5). Each nuclear pore has a central opening which provides direct continuity between the cytoplasm and the nucleus and also serves as a selective barrier for the nucleocytoplasmic exchange of macromolecules.

The configuration of the nuclear membranes is known to vary according to the state of contraction of the cardiac muscle cells.[8,9] The nuclear membranes appear smooth in contour during diastole and become wrinkled during systole. The degree of systolic wrinkling or redundancy of the nuclear membranes has been found to be proportional to the extent of sarcomere shortening.[8,9] The outer nuclear membranes of cardiac muscle cells are connected to the Z bands of the myofibrils by means of cytoskeletal filaments which measure 100 Å in diameter and differ morphologically from the actin and myosin classes of filaments.[10] These cytoskeletal filaments (Figure 5), the connections between the outer nuclear membrane and the tubules of the sarcoplasmic reticulum (Figures 3 and 4), and the basket-like network of microtubules which surround the nucleus (Figures 2 and 4) are the three structural components which maintain the nucleus in a stable position in the cardiac muscle cell.

The various nuclear membrane alterations which we observed in cardiac muscle cells were classified into three categories according to whether such changes involved the formation of: a) simple convolutions or irregularities of contour of the nuclear membranes; b) nuclear pseudoinclusions formed by the presence of cytoplasmic components in invaginations of the nuclear membranes, or c) intranuclear tubules derived from the inner nuclear membrane.

Convolutions or Irregularities of Contour of Nuclear Membranes

The nuclear membranes in nonhypertrophied or minimally hypertrophied cardiac muscle cells followed wavy courses (Figure 1), because most muscle cells in biopsy specimens were contracted. Therefore, the nuclear membranes appeared serrated in sections cut perpendicular to their surfaces, *ie*, in longitudinal sections through central areas of nuclei. In longitudinal sections which just grazed the nuclear surfaces the folds of the nuclear membranes of highly contracted cells often were evident as isolated, discontinuous areas of nuclear material which ex-

tended in a transverse direction and which, due to the highly tangential plane of sectioning, had poorly defined limiting membranes. These observations indicated that the folds of the nuclear membranes were pleat-like and were oriented perpendicular to the longitudinal axis of the cell. These folds were present only along the lateral surfaces of the nuclei. The tips of the nuclei consistently appeared smooth in contour, usually having a convex face, or less commonly, a concave one.

Alteration of shape was the most common change in the nuclei of hypertrophied cardiac muscle cells (Figures 6–11). Although present to some extent in each of the biopsies examined, this alteration was most pronounced in biopsies from patients with hypertrophic cardiomyopathy. As described in detail elsewhere,[2,3] the latter patients showed the most severe degrees of cellular enlargement and irregularity of cellular shape. Alterations of nuclear shape in hypertrophied cells (Figures 6–10) consisted of variously irregular deviations from the smooth, ovoid shape (*ie*, circular in transverse sections and oval in longitudinal sections) of nuclei in nonhypertrophied cells. These changes in overall nuclear shape frequently were accompanied by severe, localized irregularities of contour of the nuclear membranes (Figure 6). The extent of these irregularities was such that they could not be accounted for on the basis of contraction of the muscle cells. Smaller irregularities of contour of nuclear membranes often occurred in the form of focal areas of marked convolutions which showed no relationship to other nuclear or cytoplasmic structures (Figure 6). A manifestation of larger irregularities in contour of nuclear membranes was the occurrence of multiple lobulations of the nuclei (Figures 8 and 9). Owing to the plane of sectioning, these lobulations at times appeared disconnected from the main portion of the nucleus (Figure 9).

Nuclear Pseudoinclusions

Nuclear pseudoinclusions occurred as consequences of invaginations of the nuclear membranes (Figures 11–15). These invaginations measured up to 5 μ in diameter and were filled with variable numbers of mitochondria, lysosomes, lipofuscin granules and glycogen particles. In tangentially cut sections these cytoplasmic protrusions into nuclei appeared circular in outline and simulated intranuclear inclusions. The cytoplasmic pseudoinclusions differed from true intranuclear inclusions by being separated from the nucleoplasm by the outer nuclear membrane, the perinuclear cistern and the inner nuclear membrane (Figure 15). Some of these pseudoinclusions represented cytoplasmic protru-

sions into shallow, pleat-like folds of the nuclear membranes. Other pseudoinclusions resulted from large, cup- or flask-like invaginations of the nuclear membranes. This was shown by the fact that their shapes appeared round in sections cut tangential to the surface of the nucleus. Concave depressions filled with Golgi cisterns and other cytoplasmic components occasionally were present at the ends of the nuclei. When cut transversely, these depressions appeared as circular areas of cytoplasm completely surrounded by a ring-shaped nucleus (Figure 11). Because of this arrangement, these areas of cytoplasm enclosed by nuclear material also gave the erroneous impression that they were intranuclear inclusions. As in the case of the pseudoinclusions described above, these areas of cytoplasm were separated from nuclear components by the inner and outer nuclear membranes and the perinuclear cistern.

In some cardiac muscle cells the nuclear pseudoinclusions were numerous and occupied large portions of the nuclear surfaces (Figure 12). In other cells the pseudoinclusions were clustered to one side of the nucleus, the remainder of which was uninvolved. The clusters of pseudoinclusions were encountered most frequently in biopsies in which tubules derived from the inner nuclear membranes also were present (Figures 12–15). Nuclear pores were present consistently in areas of nuclear membranes involved in the formation of pseudoinclusions.

Intranuclear Tubules Derived from the Inner Nuclear Membrane

Two types of intranuclear tubules were recognized. The clinical and morphologic observations in the 6 patients in whom these tubules occurred are presented in Table 1. Both types of tubules were intranuclear; both were derived from inward extensions of the inner nuclear membrane, and both showed continuity of their lumina with the lumen of the perinuclear cistern. Cardiac muscle cells containing intranuclear tubules showed varying degrees of hypertrophy and degeneration. Such changes, however, occurred to a similar extent in adjacent cells in which intranuclear tubules were not present. The 6 patients in whom these tubules were found did not fit into a single diagnostic category and did not have clinical features that could distinguish them from other patients in whom intranuclear tubules were not present.

Tubules of the first type (Figures 12–16) were rodlike, measured from 420 to 650 Å in diameter, had well-defined points of branching or bifurcation, and extended into the depths of the nuclei, where they terminated. They occurred in clusters of up to several hundred tubules

Table 1—Clinical and Pathologic Data on 6 Patients with Intranuclear Tubules Cardiac Muscle Cells

Patient No.	Age (yrs)	Sex	Clinical diagnosis	Site from which tissue obtained	Procedure for obtaining tissue	Intranuclear tubules: Cylindrical, C-A	Intranuclear tubules: Coiled, not C-A	Dense material in perinuclear cistern	Other pathologic findings
1	46	M	Combined aortic stenosis and regurgitation	Apex of LV	Operative needle biopsy	+	+	+	Hypertrophied and degenerated CMC; interstitial fibrosis; aggregates of tubules in cytoplasm
2	56	M	Congestive cardiomyopathy and chronic alcoholism	Right side of VS	Konno catheter biopsy	+	—	+	Cellular hypertrophy; dilated sarcoplasmic reticulum and T tubules
3	10	M	Discrete sub-valvular aortic stenosis and ASH	Left side of VS	Operative resection	+	+	+	Severely degenerated CMC; marked interstitial fibrosis; disorganized arrangement of hypertrophied CMC
4	29	F	Nonobstructive ASH	Left side of VS	Operative resection	+	—	—	Disorganized arrangement of hypertrophied CMC
5	36	M	ASD; severe valvular and infundibular pulmonary stenosis	CSV	Operative resection	+	—	+	Hypertrophied and degenerated CMC; interstitial fibrosis
6	43	M	Combined aortic stenosis and regurgitation	Apex of LV	Operative biopsy	+	—	—	Hypertrophied and degenerated CMC; interstitial fibrosis; aggregates of tubules in cytoplasm

M = male, F = female, LV = left ventricle, VS = ventricular septum, ASH=asymmetric septal hypertrophy or hypertrophic cardiomyopathy, ASD = atrial septal defect, CMC = cardiac muscle cells, + = present, — = absent, CSV = crista supraventricularis, C-A = outer wall of tubule associated with marginated chromatin.

per nucleus (Figure 13). These clusters usually involved only one area of the nucleus and frequently were adjacent to nuclear pseudoinclusions (Figures 12 and 14). Remaining areas of such nuclei usually showed prominent convolutions and infoldings of their membranes. Some of these infoldings simulated intranuclear tubules, from which they differed by being associated with both the inner and the outer nuclear membranes (Figure 17). The chromatin in nuclei containing these tubules was more extensively marginated than usual, but otherwise was normal in appearance. The tubules were not associated with the nucleoli. The distinctive characteristics of these tubules were the straight, smooth, cylindrical shape of their walls and the consistent association between their outer surface (*ie*, the inner surface of the inner nuclear membrane) and a band of marginated chromatin which surrounded each tubule and measued from 200 to 300 Å in width (Figures 15 and 16). Such an association was similar to that existing between the nuclear chromatin and the inner nuclear membrane in areas not involved in the formation of intranuclear tubules. Some of these tubules had electron-lucent lumina (Figure 15). Others were filled with moderately electron-dense, finely granular material (Figure 18). In some nuclei this material also was present in wide, saccular, dilated spaces, up to 2 μ in diameter, which were formed by larger invaginations of the inner nuclear membrane (Figure 18). Some of the accumulations of this material did not show connections with the perinuclear cistern and appeared as rounded (Figure 19) or oval (Figure 20) intranuclear bodies limited by a single, trilaminar membrane (*ie*, the inner nuclear membrane). Proliferation of the outer nuclear membrane occurred extremely rarely and only in association with other, severe abnormalities of the nuclear membranes (Figure 14).

Intranuclear tubules of the second type (Figures 18 and 21) had the same diameters (400 to 650 Å) as the straight tubules described above. They were highly coiled or convoluted, showed irregular branching, usually had clear lumina, and formed rounded or irregularly shaped tangles in peripheral areas of the nucleoplasm. The membranes forming these tubules were single and trilaminar but, in contrast to those of the first type of intranuclear tubules, were not associated with marginated nuclear chromatin. These membranes were continuous with the inner nuclear membrane. This continuity was easily recognized by the fact that the area of demarcation between these two structures showed an abrupt termination of the association between marginated nuclear chromatin and the surface of the membrane. The areas of connection be-

tween the inner nuclear membrane and the membranes of the coiled intranuclear tubules sometimes were localized near the nuclear surfaces; more commonly, however, the coiled tubules appeared to be connected with straight segments of invaginated, chromatin-associated inner nuclear membrane rather than being directly connected with the inner nuclear membrane at the nuclear surfaces (Figures 18 and 21). Coiled tubules were observed only in nuclei in which straight tubules also were present.

Discussion

This study describes certain alterations which the nuclear membranes of human cardiac muscle cells undergo during the course of cardiac hypertrophy. These alterations range from foldings and convolutions, which involve both the inner and the outer nuclear membranes to the same extent, to intranuclear tubules, which result from asymmetric, selective growth of the inner nuclear membrane. It is clear from the observations reported herein that the nuclear membranes follow more tortuous and convoluted courses in hypertrophied than in normal cardiac muscle cells. The irregularities of contour of the nuclear membranes are accentuated by the contraction of muscle cells which takes place during cutting and fixation of biopsy material.[5] Nevertheless, the extent to which convolutions of nuclear membranes occur in hypertrophied cardiac muscle cells is much greater than can be accounted for on the basis of contraction artifact. These irregularities markedly increase the surface area of the nuclei and, therefore, the area available for nucleocytoplasmic interactions. Thus, it appears that the synthesis of nuclear membranes in hypertrophied cardiac muscle cells can be in excess of that needed to simply accomodate the increase in nuclear volume which occurs as nuclei grow. This excessive growth of nuclear membranes is manifested in the form of redundant folds and convolutions.

The irregularities of the nuclear surfaces in hypertrophied cardiac muscle cells lead to the formation of nuclear pseudoinclusions, which actually are globular, finger-like or flask-shaped protrusions of portions of cytoplasm into the nuclei. In histologic preparations it is difficult or impossible to distinguish between these pseudoinclusions and true intranuclear inclusions. This distinction, however, can be made readily by electron microscopy. In ultrathin sections the pseudoinclusions appear as isolated areas of cytoplasm enclosed within nuclei and limited by two membranes. The outer membrane of each inclusion is in contact with the nuclear chromatin and therefore is the inner nuclear membrane; the

inner membrane of the inclusion is in contact with its content (*ie*, cytoplasmic organelles) and is the outer nuclear membrane. Some pseudoinclusions are filled with glycogen particles and simulate intranuclear glycogen deposits. The latter can be distinguished from glycogen-rich nuclear pseudoinclusions by criteria outlined in a previous report from this laboratory.[11]

The nuclear pseudoinclusions in hypertrophied cardiac muscle cells are morphologically similar to those which occur in a large variety of other types of cells,[12,23] including certain neoplastic cells,[13,17,18,20,23] in which such deformations of the nuclear membranes cannot be attributed to artifacts produced by cellular contraction. In muscle tissues, nuclear pseudoinclusions have been described in detail only in skeletal muscle cells of Bar Harbor strain 129 dystrophic mice[19] and of rats with experimentally induced vitamin E deficiency.[16] Woodard[22] found a frequent occurrence of intranuclear cytoplasmic invaginations in mouse liver and in extraorbital lachrymal glands of rats, organs which have a high incidence of hyperploid nuclei. Woodard considered these pseudoinclusions to be related to abnormal or unusual mechanisms of cell growth. Our observations are consistent with this, since hyperploid nuclei also are found frequently in hypertrophied human hearts.

The intranuclear tubules in cardiac muscle cells represent highly unusual abnormalities in which the abnormal growth of the inner nuclear membrane is not matched by that of the outer nuclear membrane, so that the outer nuclear membrane follows its usual course while the inner nuclear membrane invaginates into the nucleoplasm. The nuclear cisterns in these areas of invaginations often contain electron-dense material of unknown composition. The two types of intranuclear tubules which we have described differ from each other in their geometry, their content and their association with nuclear chromatin. A relationship between the morphology of the tubules and nuclear chromatin was clearly evident. Tubules of the type associated with a peripheral "coating" of marginated chromatin were straight, smooth and cylindrical, whereas tubules not associated with chromatin were coiled and followed highly in irregular paths. Furthermore, some tubules showed a transition from the chromatin-associated type to the coiled type, and the coiling portions of these tubules began at the points where the association with chromatin terminated. These observations suggest: a) that certain areas of the tubular membranes have chromatin-binding loci, as do other areas of the inner nuclear membrane; b) that chromatin stabilizes the structure of the tubular walls, and c) that the two types of tubules are closely related manifestations of the same phenomenon.

In discussing the possible significance of the intranuclear tubules in cardiac muscle cells, it is useful to compare their morphologic features with those of intranuclear tubules described in other reports. Such comparisons are essentially limited to cells other than muscle, because intranuclear tubules in muscle cells have been reported in one other study.[24,25] To facilitate these comparisons, and since the subject of intranuclear tubules has not been reviewed in detail, we have classified the various types of intranuclear tubules into three groups according to the scheme outlined in Table 2. The most basic distinction between these different tubules concerns whether or not they are derived from the nuclear membranes, *ie*, whether or not continuity between the tubular walls and the nuclear membranes can be demonstrated. Tubules derived from the nuclear membranes have been subdivided into three categories, depending on whether or not they develop in association with evidence of viral invasion of the cell and depending on whether they originate from the inner or the outer nuclear membranes. Tubules not derived from the nuclear membranes constitute a heterogeneous group, as do tubules of uncertain or undetermined origin.

The two types of tubules reported in this communication belong to the group of tubules which are derived from the inner nuclear membrane and which are not associated with evidence of viral invasion of the cells. Tubules which meet these criteria also have been described in cells of Novikoff ascites hepatoma,[26,27] Taper hepatoma,[28] Yoshida ascites hepa-

Table 2—Classification of Intranuclear Tubules

I.	Tubules Derived from Nuclear Membranes
	A. Derived from invaginations of the inner nuclear membrane; not associated with evidence of viral infection
	B. Derived from invaginations either of the inner nuclear membrane or both the inner and outer nuclear membranes; associated with replication, coating and transnuclear passage of viral particles, and with reduplications of the nuclear membranes
	C. Smooth-walled, branching tubules which occupy either the perinuclear cistern or cisterns of endoplasmic reticulum and which can be derived from the outer nuclear membrane, the inner nuclear membrane or the membranes of endoplasmic reticulum
II.	Tubules Not Derived from Nuclear Membranes
	A. Microtubules similar to those normally present in the cytoplasm
	B. Myxoviruses and paramyxoviruses
	C. Myxovirus-like tubules in polymyositis
III.	Tubules of Undetermined Origin
	A. Tubules in cells transplanted into rabbits
	B. Tubules induced by estrogen in mammotroph cells of pituitary
	C. Tubules induced by ACTH in adrenal cortical cells

toma,[29,30] proliferating, preneoplastic rat hepatocytes in carcinogenesis induced by *N,N*-dimethylaminoazobenzene,[31] trophoblast III of rat and mouse chorioallantoic placenta,[32] and human endometrial epithelium.[33–46] Tubules in the various types of hepatomas mentioned above were few in number, were associated with a layer of marginated chromatin and ribonucleoprotein particles, and ranged in diameter from 500 to 3000 Å. These features are similar to those of the chromatin-associated tubules in myocardium. The nuclear tubules were more numerous and occupied larger areas of the nuclear surfaces in cardiac muscle cells than in any of the other cell types in which they have been described. The intranuclear tubules in trophoblast III of rat and mouse chorioallantoic placenta [32] differ from the chromatin-associated intranuclear tubules in cardiac muscle cells and also from the intranuclear tubules in endometrial epithelial cells,[33–46] preneoplastic hepatocytes [31] and hepatoma cells [26,30] by not being associated with condensations of chromatin or ribonucleoprotein particles against their outer walls. In this respect, the tubules in trophoblast III nuclei resemble the coiled tubules in nuclei of cardiac muscle cells. The latter tubules, however, are much more convoluted than those in trophoblast III nuclei. The pattern of arrangement of the intranuclear tubules in trophoblast III is reminiscent of that in the intranuclear tubules of human endometrial epithelial cells. First described by Daubrauszky and Pohlmann,[33–35] the tubules in human endometrial epithelium develop only during the postovulatory half of the menstrual cycle and are subject to specific hormonal influences.[36–46] Because of their association with nucleoli, these tubules have been variously named intranuclear corpuscles,[38] nucleolar channels,[39] nucleolar canalicular systems [41] and nucleolar baskets.[36] More *et al* [46] have described these tubules as forming a "nucleolar channel system" which consists of a hollow, spherical stack of interdigitating tubules. These tubules measure from 600 to 1000 Å in diameter, arise from the inner nuclear membrane, form a nine-turn spiral and terminate bluntly. Toward the end of the menstrual cycle, the nuclear tubules in human endometrial epithelium form a disordered mass which often takes the form of a nuclear protrusion.[46] Evidence presented by More *et al* suggests that these tubules are extruded and incorporated into giant lysosomes.[46] The tubules in rat trophoblast III (which undergoes degeneration as parturition approaches) sometimes were found in close approximation to cytoplasmic membranous whorls that invaginated into the nuclei and structurally resembled lysosomes.[32] Luginbuhl [42] and Kohorn *et al* [43,44] showed that the formation of intranuclear tubules in organ cultures of human proliferative endometrium can be induced by

progesterone, medroxyprogesterone acetate, chlormadinone, 19-progesterone and 19-chlormadinone, but not by estradiol, testosterone or compounds of the group of 19-nortestosterone progestational steroids (*ie*, norethindrone, norethynodrel, dimethisterone, norgestrel or ethynodiol diacetate). Kohorn *et al* interpreted these data as indicating that the acyl group in the 17-β position of the D ring of the progestational steroid is necessary to induce the formation of endometrial intranuclear tubules.[44] These observations show that the inner nuclear membranes in certain cell types can show discrete, well-defined proliferative responses to chemical stimuli.

Discrete responses of the nuclear membranes to other stimuli also are evident in the changes which these structures undergo in cells infected with viruses of the herpes,[47–54] adenovirus [55,56] and cytomegalovirus [57–66] groups, which proliferate within nuclei. Cells infected with these agents show: a) intranuclear viral particles; b) intranuclear tubules which are derived either from the inner nuclear membrane or from both the inner and the outer nuclear membrane, are closely associated with viral particles, and can have single, double or multiple walls, or spiral configurations; and c) multiple lamellar structures which are formed by reduplications and foldings of the nuclear membranes. We did not observe viral particles or lamellar structures derived from reduplication of nuclear membranes in any of the 134 cardiac biopsies. Therefore, we do not believe that the intranuclear tubules which we have observed represent evidence of cardiac viral infection.

The intranuclear tubules in cardiac muscle cells also differ from the smooth-walled, branching tubules (tubuloreticular structures) which arise from invaginations of the membranes of nuclei or endoplasmic reticulum. These smooth-walled tubules have been observed in glomerular and capillary endothelial cells and in lymphocytes of patients with systemic lupus erythematosus and other autoimmune diseases, in various types of neoplastic cells, and in association with numerous viral infections (these have been reviewed by several authors [67–72]). These tubules do not appear to be composed of viral particles. Although they have been considered to have a similarity to myxo- or paramyxoviruses,[70] recent studies [71] have shown that they differ morphologically and histochemically from these viruses. These tubules occur in aggregates and have small diameters (200 to 300 Å). Their pattern of branching varies considerably; they can form highly regular, crystalline aggregates.[67] The smooth-walled, branching tubules usually are located within cisterns of endoplasmic reticulum or within the perinuclear cistern,[68–72] but they are also seen within nuclei.[73–75] In myocardium these tubules have been

reported in the cytoplasm of capillary endothelial cells of 3 patients: 1 with Coxsackie viral myocarditis,[68] 1 with thyrotoxic heart disease and thyrotoxic myopathy [5] and 1 with polymyositis, chronic alcoholism and congestive cardiac failure.[5] The smooth-walled, branching tubules just described may be regarded as representing another type of proliferative response of the membranes of nuclei and endoplasmic reticulum either to injury or to viral infection. It must be remembered that the outer nuclear membrane is continuous with the membranes of the endoplasmic reticulum, and that proliferation of normal-appearing (nonaggregated) tubules of endoplasmic reticulum occurs, particularly in liver cells,[76–79] as a response to the administration of a variety of drugs. Proliferation of smooth tubules of sarcoplasmic reticulum is a common finding in degenerated cardiac muscle cells of patients with severe cardiac hypertrophy.[4] Furthermore, we found aggregates of tubules derived from sarcoplasmic reticulum [72] in the cytoplasm of cardiac muscle cells in 2 of the 6 patients who also had intranuclear tubules. The pathogenesis of these changes in cardiac muscle cells is unclear.

Two types of intranuclear tubules which are not constituted by invaginations of the nuclear membranes are: a) microtubules of the type ordinarily found in the cytoplasm, and b) myxoviruses and paramyxoviruses. The presence of microtubules in interphase nuclei (as opposed to dividing nuclei, where microtubules normally form part of the mitotic apparatus) has been reported as a rarity.[80,81] These microtubules measure about 200 Å in diameter and may form complex intranuclear aggregates (see Smith and Smith [80] for review) similar to those which develop in the cytoplasm of vincristine-treated cells.[82] The intranuclear tubules in cardiac muscle cells differ clearly from intranuclear microtubules and from the tubular structures which have been found in the cytoplasm and nuclei of a variety of types of cells infected with myxoviruses and paramyxoviruses.[83–90] These tubular structures represent aggregates of viral particles (rather than extensions of the nuclear membranes), show an axial periodicity of approximately 115 Å, and are much smaller (approximately 200 Å in diameter) than the intranuclear tubules in cardiac muscle cells. The myxovirus-like tubules observed by Chou in nuclei and cytoplasm of skeletal muscle cells from a patient with chronic polymyositis [24,25] and by Chandra *et al* in transplanted human leukocytes [91] also differ from the intranuclear tubules in cardiac muscle cells.

Three other reports of intranuclear tubules of undetermined origin remain to be considered. The first two are additional examples of formation of intranuclear tubules as responses to hormonal agents, *ie,* induc-

tion by estradiol in mammotroph cells of pituitary gland of Mongolian gerbils [92,93] and induction by ACTH in cells of zona fasciculata of adrenal cortex of calves.[94] In both of these cell types the hormones induce the formation of smooth-walled vesicles, tubules and cisterns of various sizes in nuclei. These structures showed little or no association with nuclear chromatin. They were similar in some respects to the intranuclear tubules derived from the inner nuclear membranes in rat and mouse trophoblast III. A relationship between the intranuclear tubules and cisterns and the nuclear membranes could not be demonstrated in mammotroph or adrenal cortical cells, and the morphogenesis of the tubules in these cells remains unexplained. These tubules were very pleomorphic and showed little resemblance to those in cardiac muscle cells.

Also of uncertain origin are the nuclear tubular inclusions observed by Stoebner *et al* [95] in several types of human, canine and rat neoplastic cells, and in normal human pancreatic acinar cells, after implantation into rabbits. These tubules measured 500 Å in diameter and up to 6800 Å in length, were straight or curved, were not associated with marginated chromatin, and showed no branching. Some tubules were surrounded by single, outer sleeves which measured 650 Å in diameter and had periodic striations (80 Å spacing) oriented obliquely or perpendicular to the longitudinal axes of the tubules. A relationship between these tubules and the nuclear membranes was not described. Of possible interest with respect to these tubules is the finding of intranuclear rod-shaped viral particles having outer sleeves with a somewhat similar substructure in midgut cells of *Gyrinus*.[81] The rod-shaped viral particles [81] and the tubules described by Stoebner [95] are morphologically different from the tubules in cardiac muscle cells.

It is evident from the observations in this study and from the reports reviewed above that nuclear membranes respond to abnormal stimuli in a number of morphologically different ways, some of which are highly specific. Synthesis of excessive amounts of nuclear membrane material and formation of nuclear pseudoinclusions appear to be responses to the stimulus of cardiac hypertrophy. It is uncertain whether the intranuclear tubules in cardiac muscle cells represent an extreme form of this response or a specific reaction to other, unknown factors.

References

1. Jones M, Ferrans VJ, Morrow AG, Roberts WC: Ultrastructure of crista supraventricularis muscle in patients with congenital heart diseases associated with right ventricular outflow tract obstruction. Circulation (In press)

2. Ferrans VJ, Morrow AG, Roberts WC: Myocardial ultrastructure in idiopathic hypertrophic subaortic stenosis: A study of operatively excised left ventricular outflow tract muscle in 14 patients. Circulation 45:769–792, 1972
3. Maron BJ, Ferrans VJ, Henry WL, Clark CE, Redwood DR, Roberts WC, Morrow AG, Epstein SE: Differences in distribution of myocardial abnormalities in patients with obstructive and non-obstructive asymmetric septal hypertrophy (ASH): Light and electron microscopic findings. Circulation 50:436–446, 1974
4. Maron BJ, Ferrans VJ, Roberts WC: Myocardial ultrastructure in patients with chronic aortic valvular disease. Am J Cardiol (In press)
5. Ferrans VJ, Massumi RA, Shugoll GI, Ali N, Roberts WC: Ultrastructural studies of myocardial biopsies in 45 patients with obstructive or congestive cardiomyopathy. Recent Advances in Studies on Cardiac Structure and Function, Vol II, The Cardiomyopathies. Edited by E Bajusz, G Rona, AJ Brink, A Lochner. Baltimore, University Park Press, 1973, pp 231–272
6. Freeman JA, Spurlock BO: A new epoxy embedment for electron microscopy. J Cell Biol 13:437–443, 1962
7. Fawcett DW, McNutt NS: The ultrastructure of the cat myocardium. I. Ventricular papillary muscle. J Cell Biol 42:1–45, 1969
8. Bloom S, Cancilla PA: Conformational changes in myocardial nuclei of rats. Circulation Res 24:189–196, 1969
9. Bloom S: Structural changes in nuclear envelopes during elongation of heart muscle cells. J Cell Biol 44:218–222, 1970
10. Ferrans VJ, Roberts WC: Intermyofibrillar and nuclear-myofibrillar connections in human and canine myocardium: An ultrastructural study. J Mol Cell Cardiol 5:247–257, 1973
11. Ferrans VJ, Maron BJ, Buja LM, Ali N, Roberts WC: Intranuclear glycogen deposits in human cardiac muscle cells: Ultrastructure and cytochemistry. J Mol Cell Cardiol (In press)
12. Kleinfeld RG, Greider MH, Frajola WJ: Electron microscopy of intranuclear inclusions found in human and rat liver parenchymal cells. J Biophys Biochem Cytol 2 (Suppl):435–438, 1956
13. Leduc EH, Wilson JW: An electron microscope study of intranuclear inclusions in mouse liver and hepatoma. J Biophy Biochem Cytol 6:427–430, 1959
14. Cossel L: Elektronenmikroskopische Befunde an "Einschlusskörpern" in den Kernen menschlicher Leberepithelzellen. Z Zellforsch 57:512–519, 1962
15. Cossel L: Elektronmikroskopische Befunde an den sog: Lochkernen in menschlichen Leberepithelzellen. Frankfurter Z Pathol 72:115–122, 1962
16. Howes EL, Price HM, Blumberg JM: The effects of a diet producing lipochrome pigment (ceroid) on the ultrastructure of skeletal muscle in the rat. Am J Pathol 45:599–631, 1964
17. Robertson DM: Electron microscopic studies of nuclear inclusions in meningiomas. Am J Pathol 45:835–848, 1964
18. Robertson DM, MacLean JD: Nuclear inclusions in malignant gliomas. Arch Neurol 13:287–296, 1965
19. Banker BQ. A phase and electron microscopic study of dystrophic muscle. I. The pathological changes in the two-week-old Bar Harbor 129 dystrophic mouse. J Neuropathol Exp Neurol 26:259–275, 1967

20. Sobel HJ: Cytoplasmic invaginations resembling nuclear inclusions. Arch Pathol 85:114–115, 1968
21. Yasuzumi G, Tsubo I, Okada K, Terawaki A, Enomoto Y: The fine structure of nuclei as revealed by electron microscopy. V. Intranuclear inclusion bodies in hepatic parenchymal cells in cases of serum hepatitis. J Ultrastruct Res 23:321–332, 1968
22. Woodard JC: Reply to letter on nuclear inclusions. Arch Pathol 85:568, 1968
23. Sobel HJ, Schwarz R, Marquet E: Nonviral nuclear inclusions. I. Cytoplasmic invaginations. Arch Pathol 87:179–192, 1969
24. Chou SM: Myxovirus-like structures in a case of human chronic polymyositis. Science 158:1453–1455, 1967
25. Chou SM: Myxovirus-like structures and accompanying nuclear changes in chronic polymyositis. Arch Pathol 86:649–658, 1968
26. Novikoff AB: A transplantable rat liver tumor induced by 4-dimethylaminoazobenzene. Cancer Res 17:1010–1027, 1957
27. Babai F, Tremblay G, Dumont A: Intranuclear and intranucleolar tubular structures in Novikoff hepatoma cells. J Ultrastruct Res 28:125–130, 1969
28. Fritzler MJ, Church RB, Wagenaar EB: Ultrastructure of Taper hepatoma ascites cells. J Electron Microsc (Tokyo) 22:73–90, 1973
29. Hoshino M: The deep invagination of the inner nuclear membrane into the nucleoplasm in the ascites hepatoma cells. Exp Cell Res 24:606–609, 1961
30. Locker J, Goldblatt PJ, Leighton J: Some ultrastructural features of Yoshida ascites hepatoma. Cancer Res 28:2039–2050, 1968
31. Karasaki S: Passage of cytoplasmic lipid into interphase nuclei in preneoplastic rat liver. J Ultrastruct Res 42:463–478, 1973
32. Carlson EC, Ollerich DA: Intranuclear tubules in trophoblast III of rat and mouse chorioallantoic placenta. J Ultrastruct Res 28:150–160, 1969
33. Dubrauszky V, Pohlmann G: Veränderungen der submikroskopischen Struktur von Drüsenzellen der Corpusmucosa während des Zyklus. Proceedings of the European Regional Conference on Electron Microscopy, Delft, 1960, Vol II. Edited by AL Houwink, BJ Spit. The Hague, NV Drukkerij Trio, 1960, pp 862–865
34. Dubrauszky V, Pohlmann G: Strukturveränderungen am Nukleolus von Korpusendometriumzellen während der Sekretionsphase. Naturwissenschaften 47:523–524, 1960
35. Dubrauszky V, Pohlmann G: Die Ultrastruktur des Korpusendometriums während des Cyclus. Arch Gynäkol 196:180–199, 1961
36. Clyman MJ: A new structure observed in the nucleolus of the human endometrial epithelial cell. Am J Obstet Gynecol 86:430–432, 1963
37. Moricard R, Moricard F: Modifications cytoplasmiques et nucleaires ultrastructurales utérines au cours de l'état folliculolutéinique á glycogène massif (Mitochondries-glycogène-nucléolonéma-chromatine sexuelle-formations tubulaires nucléaires). Gynecol Obstet (Paris) 63:203–220, 1964
38. Ancla M, de Brux J, Belaisch J, Musset R: Influence de l'équilibre oestrogène-progestérone sur les ultrastructures de l'endomètre humain. I. Morphologie et évolution de corpuscules intra-nucléaires présents dans les cellules glandulaires de l'endomètre sous l'effet de la norethistérone et dans les stérilités essentielles. Gynecol Obstet (Paris) 63:239–248, 1964

39. Terzakis JA:The nucleolar channel system of human endometrium. J Cell Biol 27:293–304, 1965
40. Ancla M, de Brux J: Occurrence of intranuclear tubular structures in the human endometrium during the secretory phase, and of annulate lamellae in hyperestrogenic states. Obstet Gynecol 26:23–33, 1965
41. Roberts DK: Canalicular structures in the nucleus of human endometrium: A three-dimensional study. J Cell Biol 35:114A, 1967
42. Luginbuhl WH: Electron microscopic study of the effects of tissue culture on human endometrium. Am J Obstet Gynecol 102:192–201, 1968
43. Kohorn EI, Rice SI, Gordon M: In vitro production of nucleolar channel system by progesterone in human endometrium. Nature 228:671–672, 1970
44. Kohorn EI, Rice SI, Hemperly S, Gordon M: The relation of the structure of progestational steroids to nucleolar differentiation in human endometrium. J Clin Endocrinol 34:257–264, 1972
45. Armstrong EM, More IAR, McSeveney D, Chatfield WR: Reappraisal of the ultrastructure of the human endometrial glandular cell. J Obstet Gynaecol Br Commonw 80:446–460, 1973
46. More IAR, Armstrong EM, McSeveney D, Chatfield WR: The morphogenesis and fate of the nucleolar channel system in the human endometrial glandular cell. J Ultrastruct Res 47:74–85, 1974
47. Fawcett DW: Electron microscope observations on intracellular virus-like particles associated with the cells of the Lucké renal adenocarcinoma. J Biophys Biochem Cytol 2:725–742, 1956
48. Falke D, Siegert R, Vogell W: Elektronenmikroskopische Befunde zur Frage der Doppelmembranbildung des Herpes-simplex virus. Arch Gesamte Virusforsch 9:484–496, 1959
49. Morgan C, Rose HM, Holden M, Jones EP: Electron microscopic observations on the development of herpes simplex virus. J Exp Med 110:643–656, 1959
50. Darlington RW, Granoff A, Breeze DC: Viruses and renal carcinoma of Rana pipiens. II. Ultrastructural studies and sequential development of virus isolated from normal and tumor tissue. Virology 29:149–156, 1966
51. Shipkey FH, Erlandson RA, Bailey RB, Babcock VI, Southam CM: Virus biographies. II. Growth of herpes simplex virus in tissue culture. Exp Mol Pathol 6:39–67, 1967
52. Chou SM, Cherry JD: Ultrastructure of Cowdry type A inclusions. I. In human herpes simplex encephalitis. Neurology 17:575–586, 1967
53. Stackpole CW, Mizell M: Electron microscopic observations in herpes-type virus-related structures in the frog renal adenocarcinoma. Virology 36:63–72, 1968
54. Nii S, Morgan C, Rose HM: Electron microscopy of herpes simplex virus. II. Sequence of development. J Virol 2:517–536, 1968
55. Morgan C, Howe C, Rose HM, Moore DH: Structure and development of viruses observed in the electron microscope. IV. Viruses of the RI-APC group. J Biophys Biochem Cytol 2:351–360, 1956
56. Gregg MB, Morgan C: Reduplication of nuclear membranes in HeLa cells infected with adenoviruses. J Biophys Biochem Cytol 6:539–540, 1959
57. Stern H, Friedmann I: Intranuclear formation of cytomegalic inclusion disease virus. Nature 188:768–770, 1960

58. Ruebner BH, Miyai K, Slusser RJ, Wedemyer P, Medearis DN: Mouse cytomegalovirus infection: An electron microscopic study of hepatic parenchymal cells. Am J Pathol 44:799–821, 1964

59. Ruebner BH, Hirano T, Slusser RJ, Medearis DN: Human cytomegalovirus infection: Electron microscopic and histochemical changes in cultures of human fibroblasts. Am J Pathol 46:477–496, 1965

60. Patrizi G, Middelkamp JN, Herweg JC, Thornton HK: Human cytomegalovirus: Electron microscopy of a primary viral isolate. J Lab Clin Med 65: 825–838, 1965

61. McGavran MH, Smith MG: Ultrastructural, cytochemical and microchemical observations on cytomegalovirus (salivary gland virus) infection of human cells in tissue culture. Exp Mol Pathol 4:1–10, 1965

62. Patrizi G, Middelkamp JN, Reed CA: Reduplication of nuclear membranes in tissue-culture cells infected with guinea-pig cytomegalovirus. Am J Pathol 50:779–790, 1967

63. Middelkamp JN, Patrizi G, Reed CA: Light and electron microscopic studies on the guinea-pig cytomegalovirus. J Ultrastruct Res 18:85–105, 1967

64. Berezeski IK, Grimley PM, Tyrrell SA, Rabson AS: Ultrastructure of a rat cytomegalovirus. Exp Mol Pathol 14:337–349, 1971

65. Chopra HC, Lloyd BJ Jr, Ablashi DV, Armstrong GR: Morphologic studies of a cytomegalovirus isolated from an owl monkey.J Natl Cancer Inst 48: 1333–1340, 1972

66. Wilson NJ, Schneider JF, Rosen M, Belisle EH: Ultrastructural pathology of murine cytomegalovirus infection in cultured mouse nervous system tissue. Am J Pathol 74:467–480, 1974

67. Chandra S: Undulating tubules associated with endoplasmic reticulum in pathologic tissues. Lab Invest 18:422–428, 1968

68. Haas JE, Yunis EJ: Viral crystalline arrays in human Coxsackie myocarditis. Lab Invest 23:442–446, 1970

69. Uzman BG, Saito H, Kasac M: Tubular arrays in the endoplasmic reticulum in human tumor cells. Lab Invest 24:492–498, 1971

70. Györkey F, Sinkovics JG, Min KW, Györkey P: A morphologic study on the occurrence and distribution of structures resembling viral nucleocapsids in collagen diseases. Am J Med 53:148–158, 1972

71. Schaff Z, Heine U, Dalton AJ: Ultramorphological and ultracytochemical studies on tubuloreticular structures in lymphoid cells. Cancer Res 32:2696–2706, 1972

72. Maron BJ, Ferrans VJ: Aggregates of tubules in human cardiac muscle cells. J Mol Cell Cardiol 6:249–264, 1974

73. Jenson AB, Spjut HJ, Smith MN, Rapp F: Intracellular branched tubular structures in osteosarcoma: An ultrastructural and serological study. Cancer 27:1440–1448, 1971

74. Schmitt D, Thivolet J, Perrot H, Tack JL, Germain D: Aspects ultrastructuraux des inclusions de type viral dans les lesions cutanées du lupus aigu et du dupus discoïde fixe. Arch Dermatol Forsch 241:317–328, 1971

75. Nieland NW, Hashimoto K, Masi AT: Microtubular inclusions in normal skin of systemic lupus erythematosus patients. Arthritis Rheum 15:193–200, 1972

76. Orrenius S, Ericsson JLE: On the relationship of liver glucose-6-phosphatase

to the proliferation of endoplasmic reticulum in phenobarbital induction. J Cell Biol 31:243–256, 1966

77. Hutterer F, Schaffner F, Klion FM, Popper H: Hypertrophic, hypoactive smooth endoplasmic reticulum: A sensitive indicator of hepatotoxicity exemplified by Dieldrin. Science 161:1017–1019, 1968
78. Emans JB, Jones AL: Hypertrophy of liver cell smooth surfaced reticulum following progesterone administration. J Histochem Cytochem 16:561–571, 1968
79. Dallner G, Bergstrand A, Nilsson R: Heterogeneity of rough-surfaced liver microsomal membranes of adult phenobarbital-treated, and newborn rats. J Cell Biol 38:257–276, 1968
80. Smith U, Smith DS: A microtubular complex in the epidermal nucleus of an insect, *Carausius morosus*. J Cell Biol 26:961–967, 1965
81. Gouranton J: Development of an intranuclear nonoccluded rod-shaped virus in some midgut cells of an adult insect, *Gyrinus natator* L. (Coleoptera) J Ultrastruct Res 39:281–294, 1972
82. Bensch KG, Malawista SE: Microtubular crystals in mammalian cells. J Cell Biol 40:95–107, 1969
83. Bouteille M, Fontaine C, Vedrenne C, Delarue J: Sur un cas d'encephalite subaiguë a inclusions: Étude anatomo-clinique et ultrastructurale. Rev Neurol 113:454–458, 1965
84. Tellez-Nagel I, Harter DH: Subacute sclerosing leukoencephalitis: Ultrastructure of intranuclear and intracytoplasmic inclusions. Science 154:899–900, 1966
85. Perier O, Vanderhaegen JJ: Indications etiologiques apportées par la microscopie électronique dans certaines encephalites humaines. Rev Neurol 115: 250–254, 1966
86. Matsumoto N: Studies on measles virus in tissue culture. II. Electron microscopic study of measles virus-infected cells and localization of virus antigen examined by ferritin-conjugated antibody method. Bull Yamaguchi Med School 13:167–189, 1966
87. Herndon RM, Rubinstein LJ: Light and electron microscopy observations on the development of viral particles in the inclusions of Dawson's encephalitis (subacute sclerosing panencephalitis). Neurology 18 (Part 2):8–120, 1968
88. Harter DH, Tellez-Nagel I: Attempts to isolate SSPE agent in cell culture. Neurology 18 (Part 2):133–137, 1968
89. Nakai T, Shand FL, Howatson AF: Development of measles virus in vitro. Virology 38:50–67, 1969
90. Raine CS, Fields BN: Neurotropic virus-host relationship alterations due to variation in viral genome as studied by electron microscopy. Am J Pathol 75:119–138, 1974
91. Chandra S, Brown DE, Aldenderfer P, Garon C, Buscheck FT, Manaker RA: Morphologic and serologic studies of transplanted human leukocyte culture (M-1) cells in laboratory animals. Cancer Res 29:1829–1839, 1969
92. Nakayama I, Nickerson PA: Intranuclear inclusions in mammotrophs of the female Mongolian gerbil. Am J Anat 135:93–104, 1972
93. Nickerson PA: Induction of intranuclear inclusions by estrogen in mammotrophs of the Mongolian gerbil. J Ultrastruct Res 44:41–48, 1973

94. Weber A, Whipp S, Usenik E, Frommes S: Structural changes in the nuclear body in the adrenal zona fasciculata of the calf following the administration of ACTH. J Ultrastruct Res 11:564–576, 1964

95. Stoebner P, Seigneurin JM, Micouin C, Colomb M, Groulade J: Sur une structure cylindrique particulière observée dans les noyaux de cellules transplantées. J Microsc 17:215:218, 1973

[Illustrations follow]

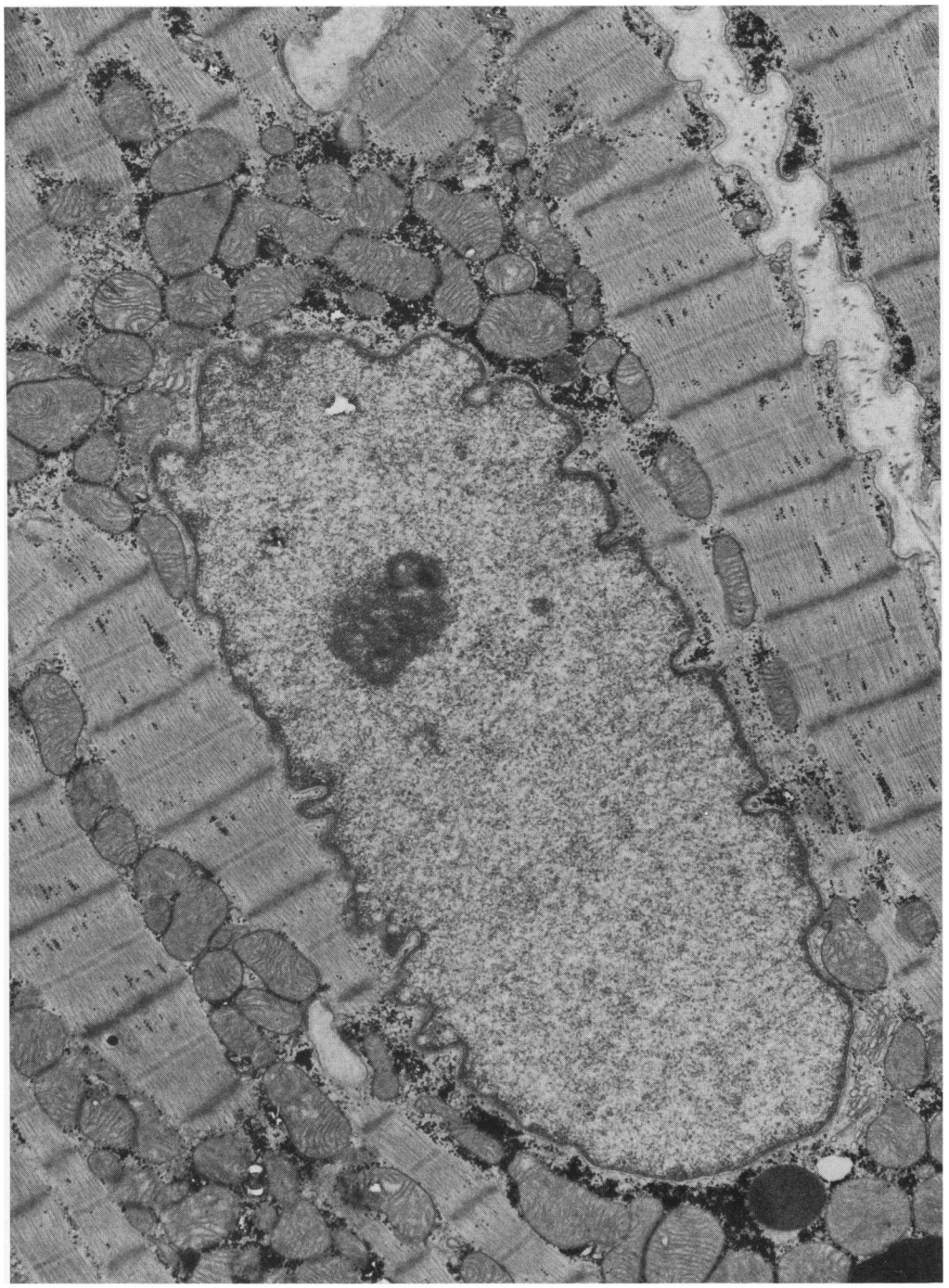


Fig 1—Part of nonhypertrophied cardiac muscle cell in crista supraventricularis from 23-year-old patient with ventricular septal defect and no obstruction to right ventricular outflow. The nucleus is oval shaped and contains a small nucleolus. The nuclear membranes show several indentations in their contour. The chromatin is finely dispersed throughout the nucleoplasm, with only a small amount of margination. The myofibrils are contracted (× 13,500).

Figures 2–5 are high magnification views of nuclear membranes in nonhypertrophied (*ie,* normal transverse diameter) cardiac muscle cells from left ventricular apical myocardium of 40-year-old woman with aortic valvular stenosis.

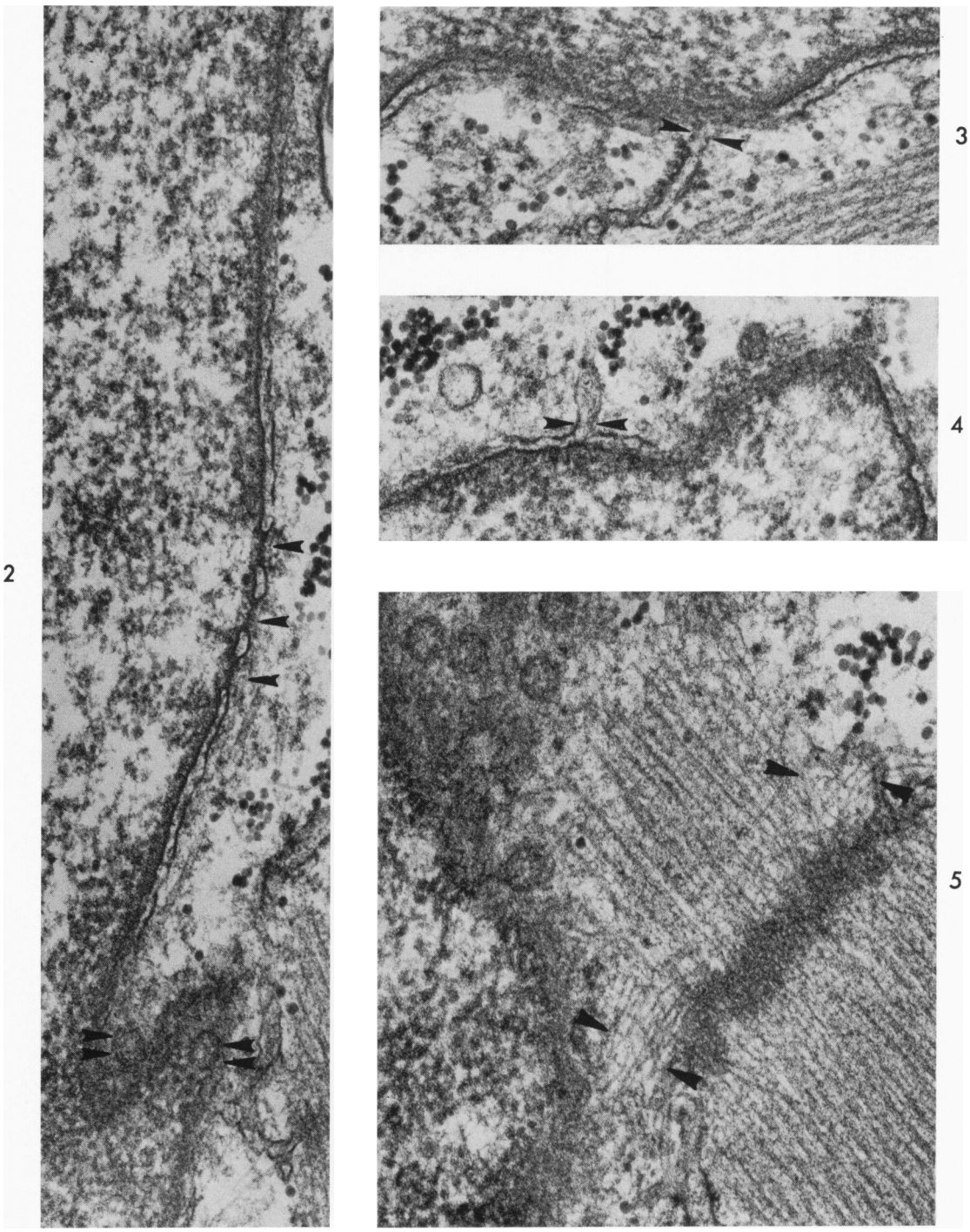


Fig 2—The inner and outer nuclear membranes follow a parallel course and are separated from one another by the perinuclear cistern. Lateral views of three closely adjacent nuclear pores are shown in the center (*single arrowheads*); *en face* views of other nuclear pores are shown in area at bottom (*paired arrowheads*), in which the nuclear membranes are sectioned tangentially. Several microtubules are in close proximity to the nuclear membranes (× 73,600). **Figs 3 and 4**—Connections (*arrowheads*) between the outer nuclear membrane and smooth-surfaced (**3**) and rough-surfaced (**4**) reticulum are shown in these views (× 82,000). **Fig 5**—Bundles of 100 Å thick filaments (*arrowheads*) extend between nuclear membrane and an adjacent Z band. Note *en face* views of several nuclear pores (× 82,000).

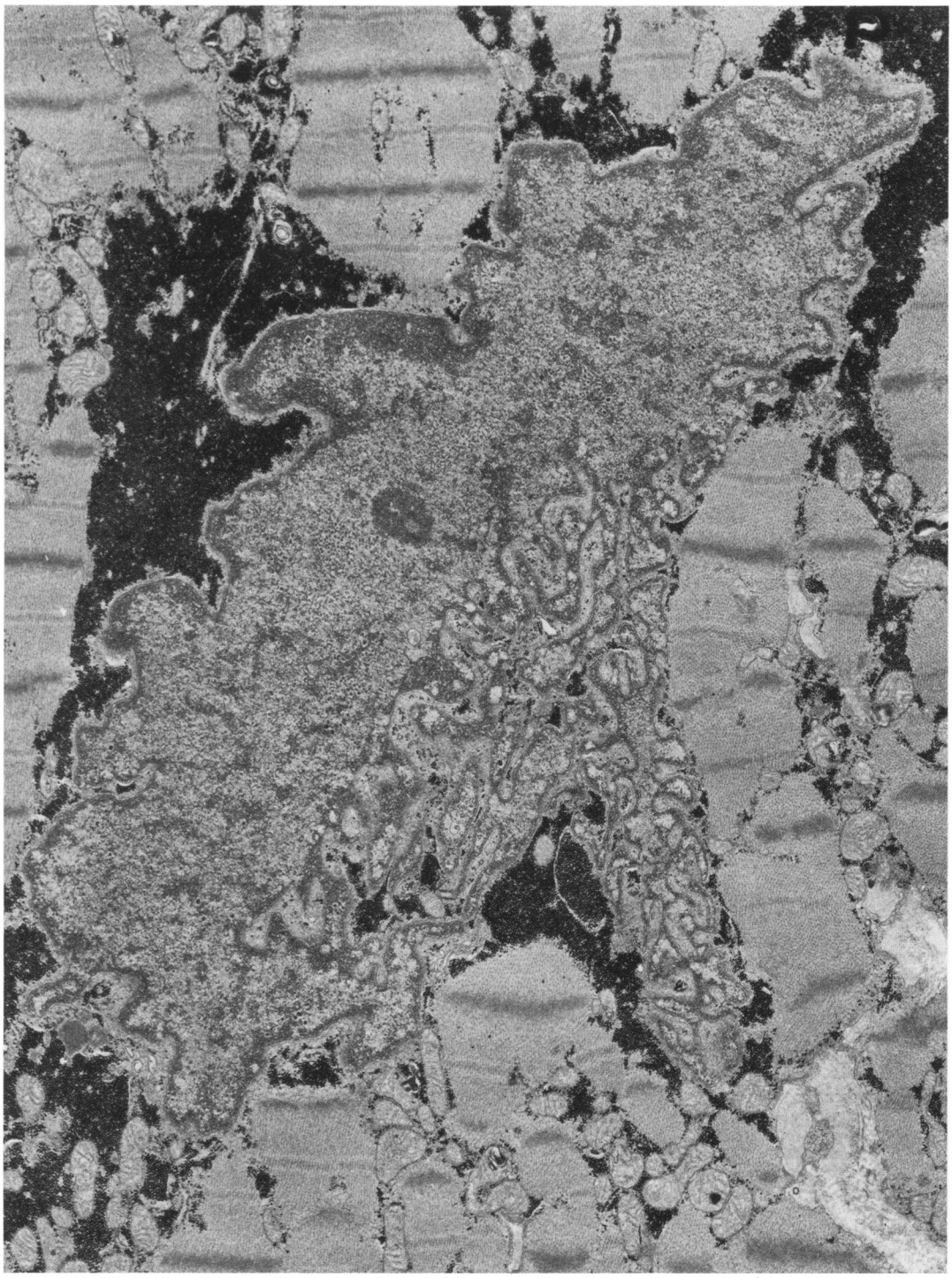


Fig 6—Nucleus of hypertrophied cardiac muscle cell shows marked irregularities of contour of one of its sides. The other side and the ends of this nucleus exhibit few convolutions. Crista supraventricularis muscle from 53-year-old man with ventricular septal defect and infundibular stenosis (× 8750).

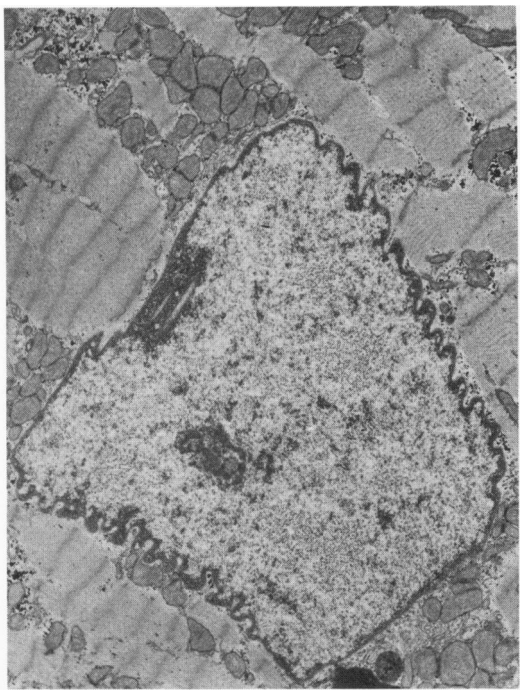
Figures 7–10 are of abnormalities of nuclear shape in hypertrophied cardiac muscle cells.

Fig 7—Blunt-tipped nucleus with mildly irregular lateral surfaces and smooth ends. Cardiac muscle from right side of ventricular septum of 46-year-old woman with thyrotoxic heart disease (× 5775).

Fig 8—Cross-section through nucleus shows asymmetric lobulations. Crista supraventricularis muscle from 36-year-old man with atrial septal defect and combined pulmonic valvular and infundibular stenosis (× 5580).

Fig 9—Irregularly shaped, highly lobulated nucleus of cardiac muscle cell from left side of ventricular septum of 47-year-old man with hypertrophic obstructive cardiomyopathy (obstructive ASH, IHSS) (× 7750).

Fig 10—Large, blunt-tipped nucleus with a concave depression at one of its ends. This depression is occupied by several large lipofuscin granules. Compare with Figure 11 which shows a section cut transversely at the level of a similar depression in the nuclear surface. Muscle from right side of ventricular septum of 40-year-old woman with chronic alcoholism and history of Coxsackie B viral myocarditis (× 10,200).



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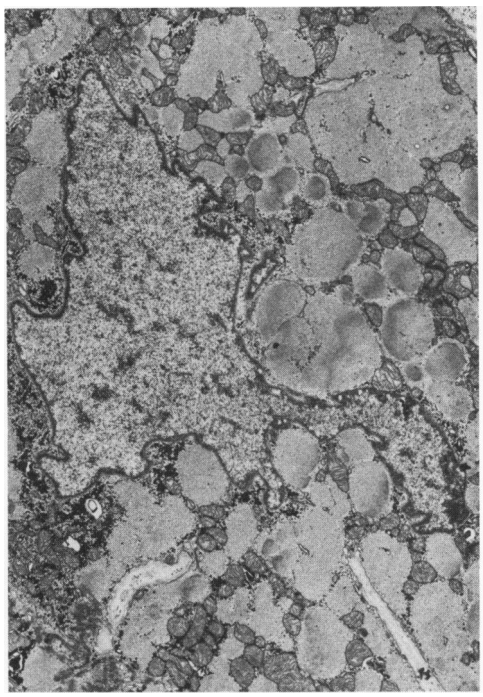

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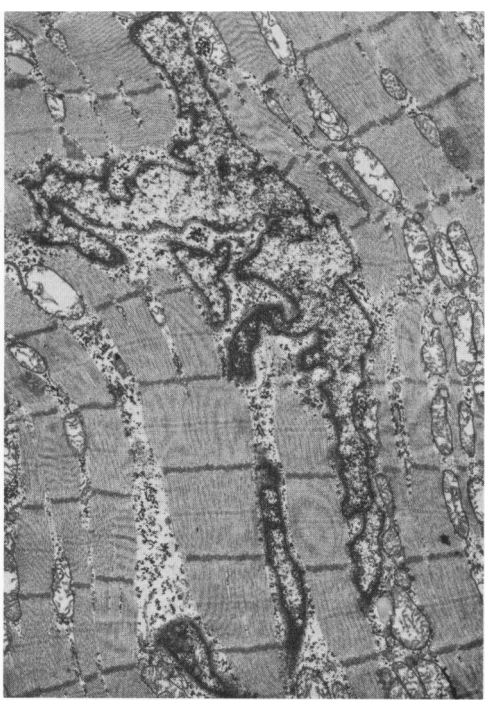

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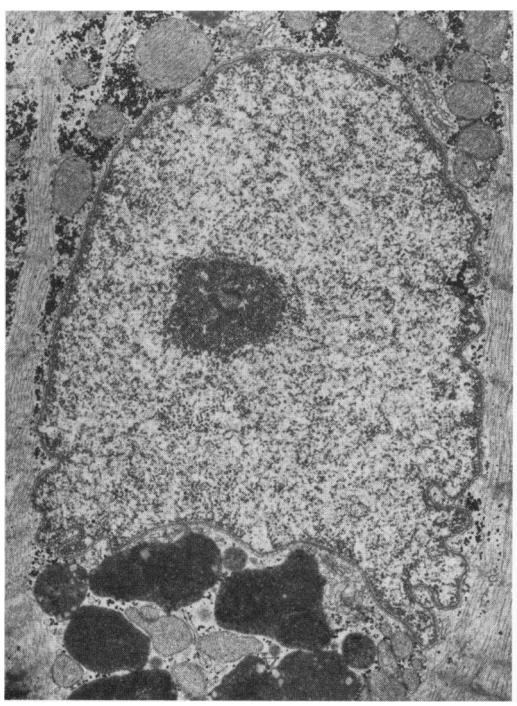

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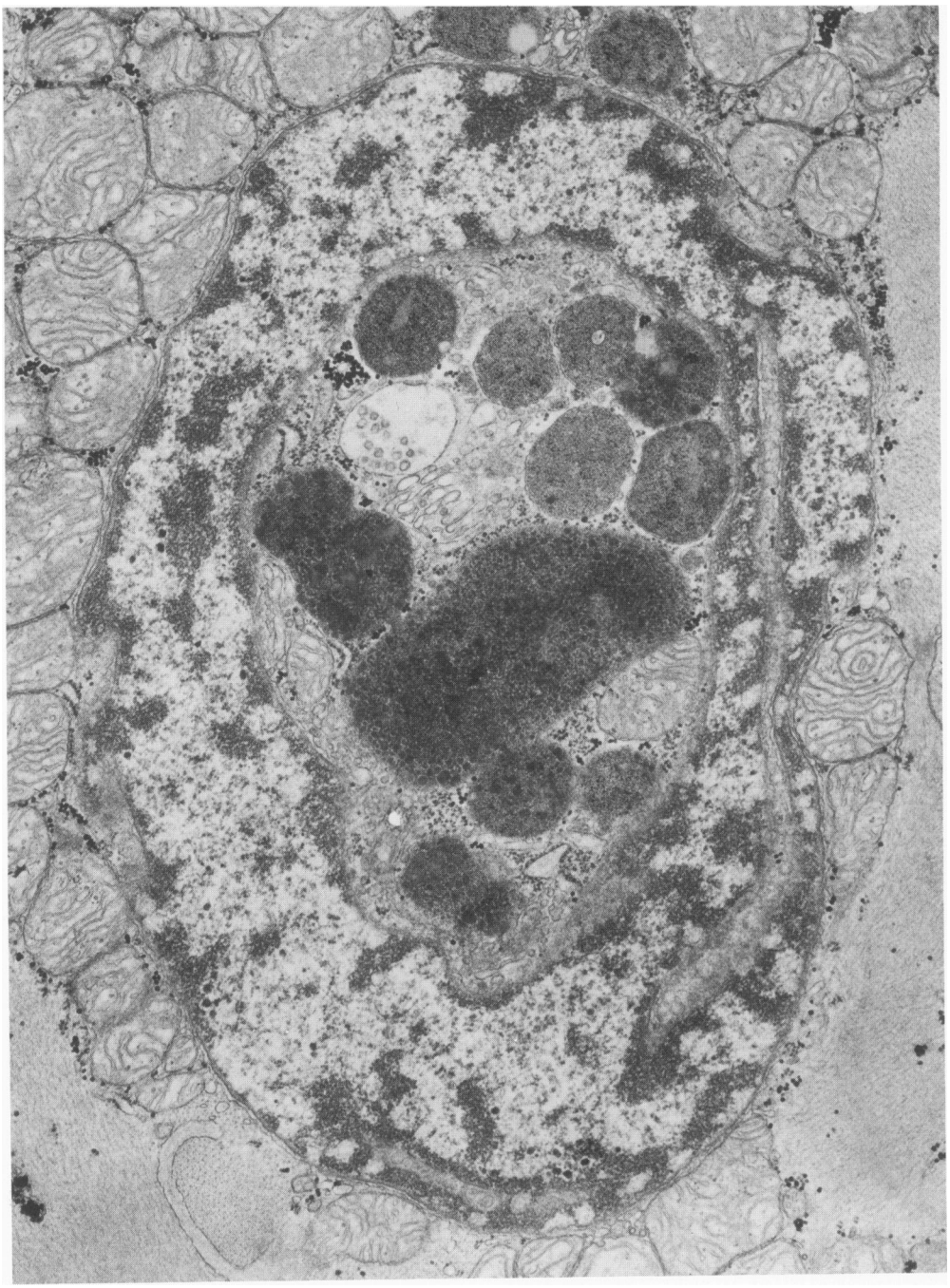


Fig 11—Large nuclear pseudoinclusion of the type shown here results from transverse section through cytoplasmic invagination at the end of nucleus of a cardiac muscle cell. Compare with Figure 10. This pseudoinclusion contains lipofuscin granules, mitochondria, glycogen, ribosomes, Golgi cisterns and a multivesicular body. Left ventricular free wall myocardium from 56-year-old man with polymyositis and congestive cardiac failure (× 20,350).

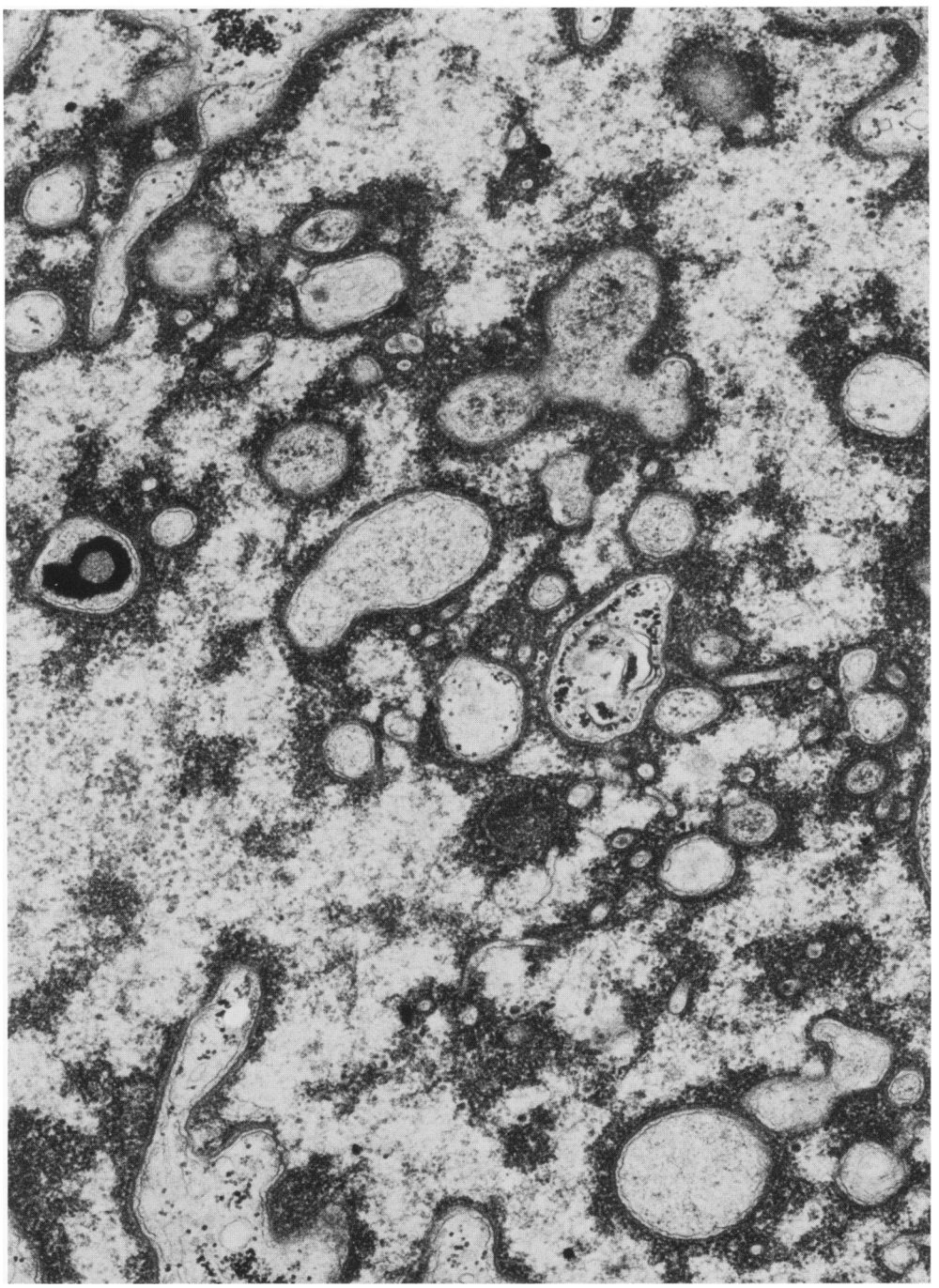


Fig 12—Tangential section through surface of nucleus of cardiac muscle cell shows large nuclear pseudoinclusions formed by invaginations of both inner and outer nuclear membranes, and small intranuclear tubules derived from cylindrical invaginations of the inner nuclear membrane. The nuclear chromatin is marginated against the inner nuclear membrane. Left ventricular apical myocardium from 46-year-old man with combined aortic stenosis and regurgitation (× 29,500).

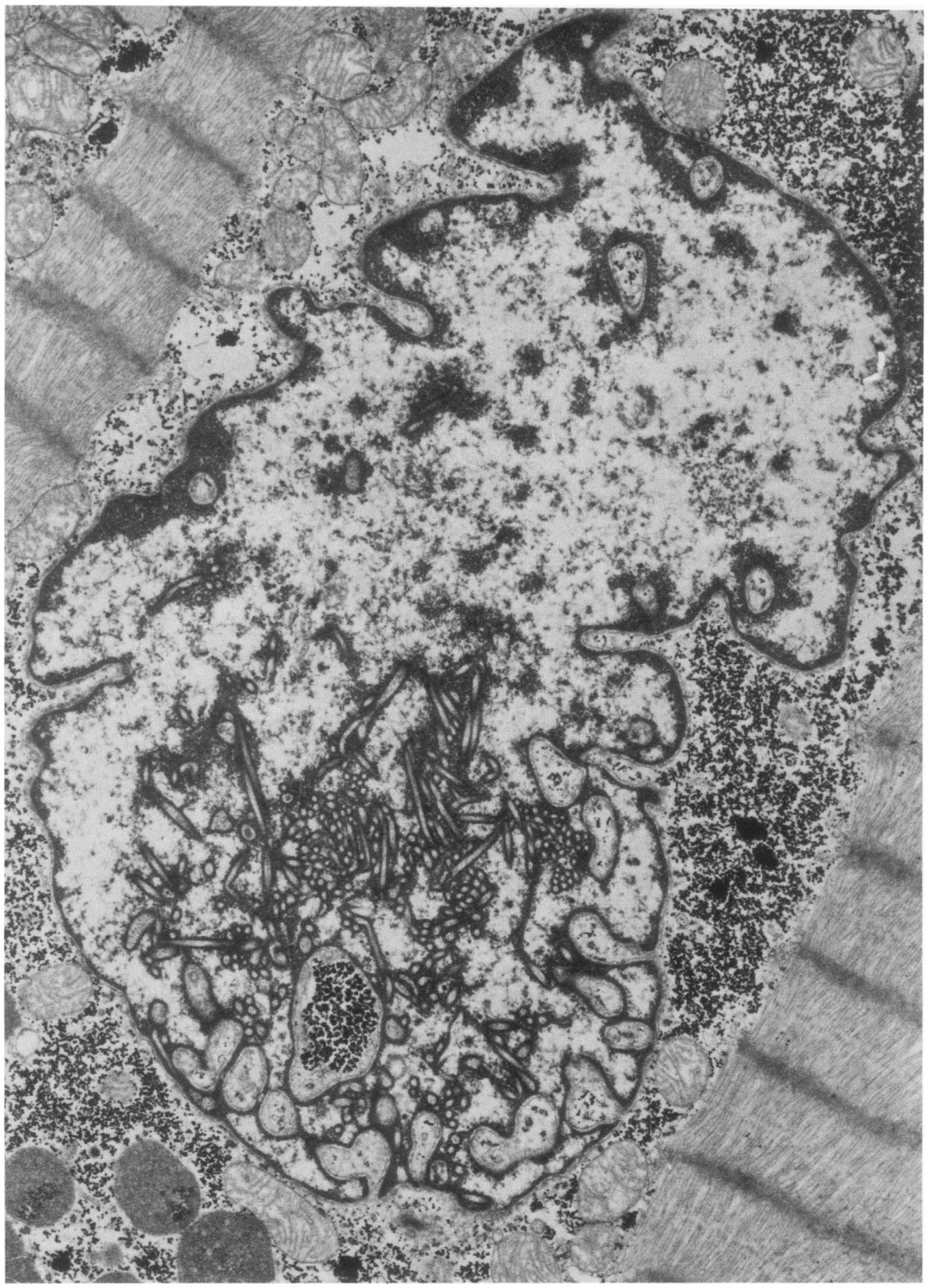


Fig 13—View of cardiac muscle cell nucleus from left side of ventricular septum of 29-year-old woman with nonobstructive hypertrophic cardiomyopathy. Numerous intranuclear tubules form a large cluster at one end of the nucleus and are associated with several nuclear pseudoinclusions, one of which is rich in glycogen (× 15,000).

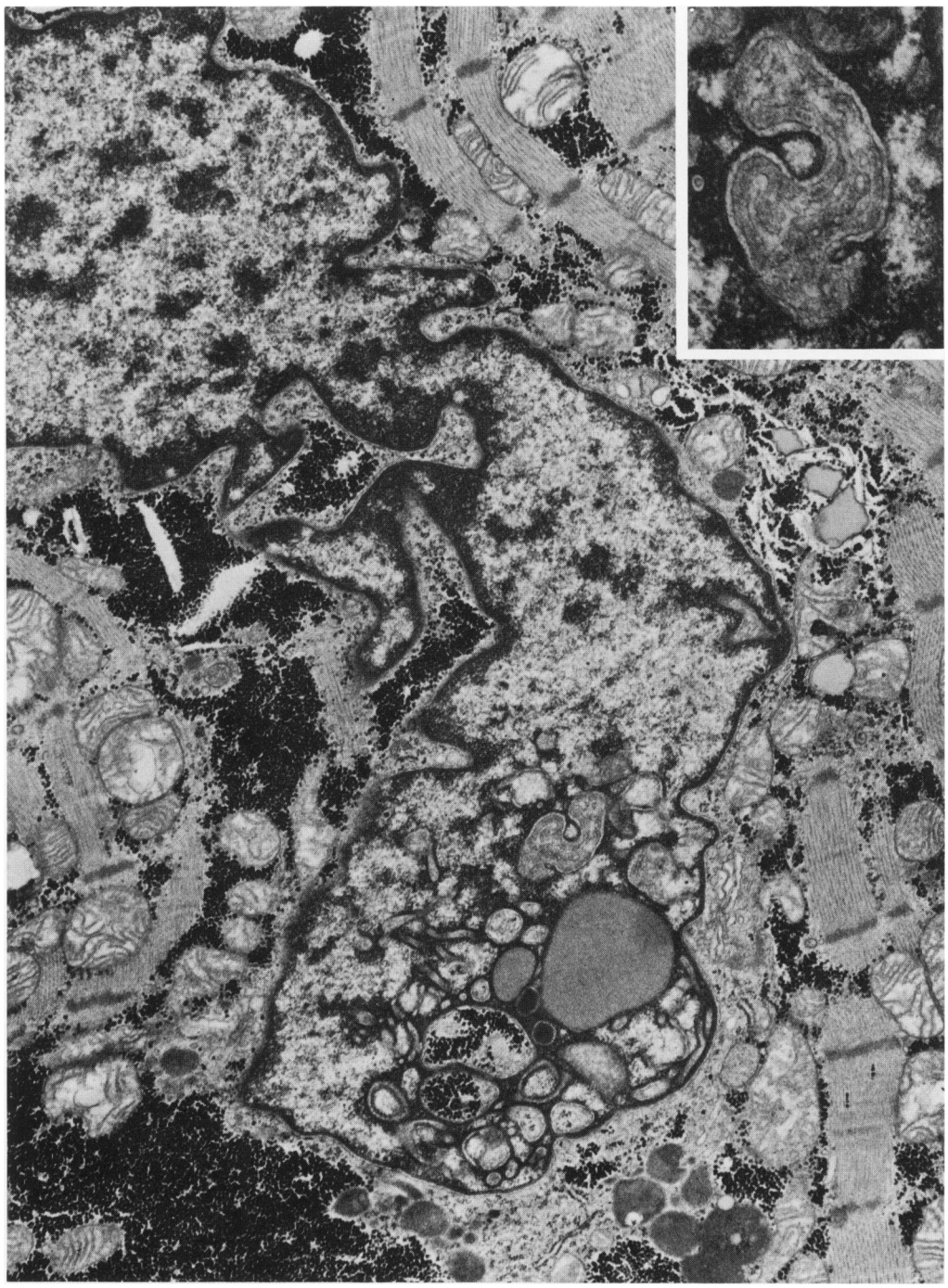


Fig 14—Part of highly convoluted, irregularly shaped nucleus of muscle cell from left side of ventricular septum of 10-year-old boy with asymmetric septal hypertrophy and discrete (fibrous ring) type of subaortic stenosis. Nucleus shows pseudoinclusions formed by cytoplasmic invaginations (compare with Figures 12 and 13), intranuclear tubules (compare with Figure 15), two inclusions formed by deposition of electron-dense material between the inner and the outer nuclear membranes (compare with Figures 18–20), and a small area of selective proliferation of the outer nuclear membrane (× 14,800). **Inset**—Higher magnification view of tubules derived from proliferation of outer nuclear membrane (× 34,800).

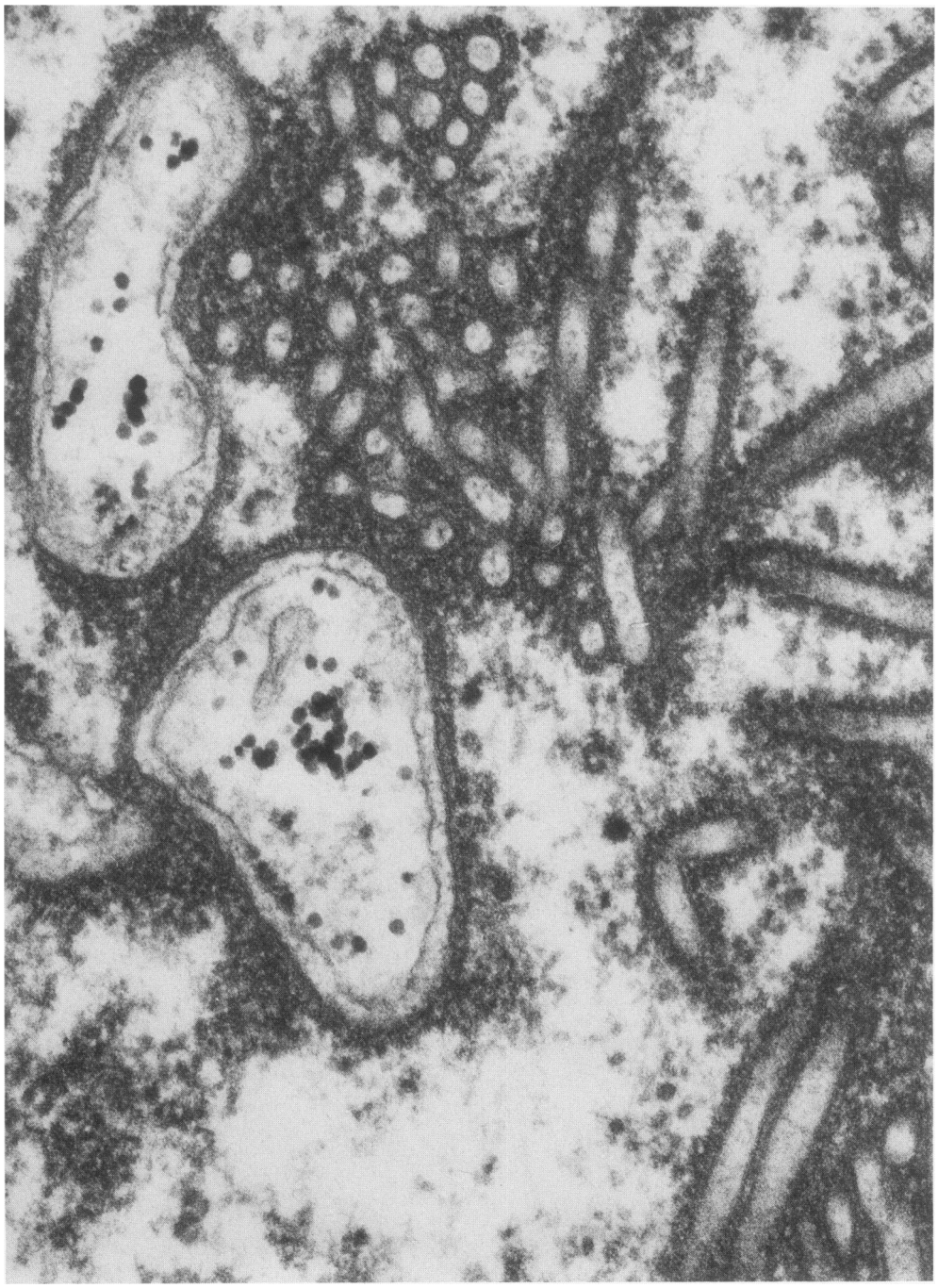
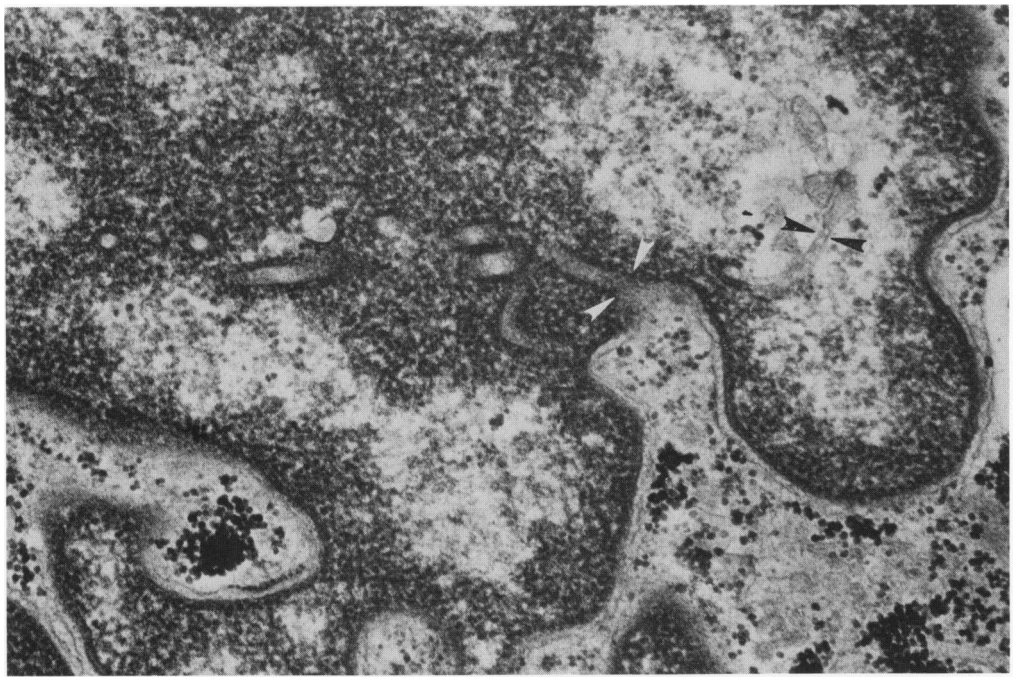


Fig 15—High magnification view of nuclear pseudoinclusions and intranuclear tubules shown in Figure 13. Two pseudoinclusions, shown on the left side of the photograph, consist of areas of cytoplasm surrounded by the outer nuclear membrane, the perinuclear cistern and the inner nuclear membrane. The intranuclear tubules have single, trilaminar membranes which are associated with chromatin (× 93,750).



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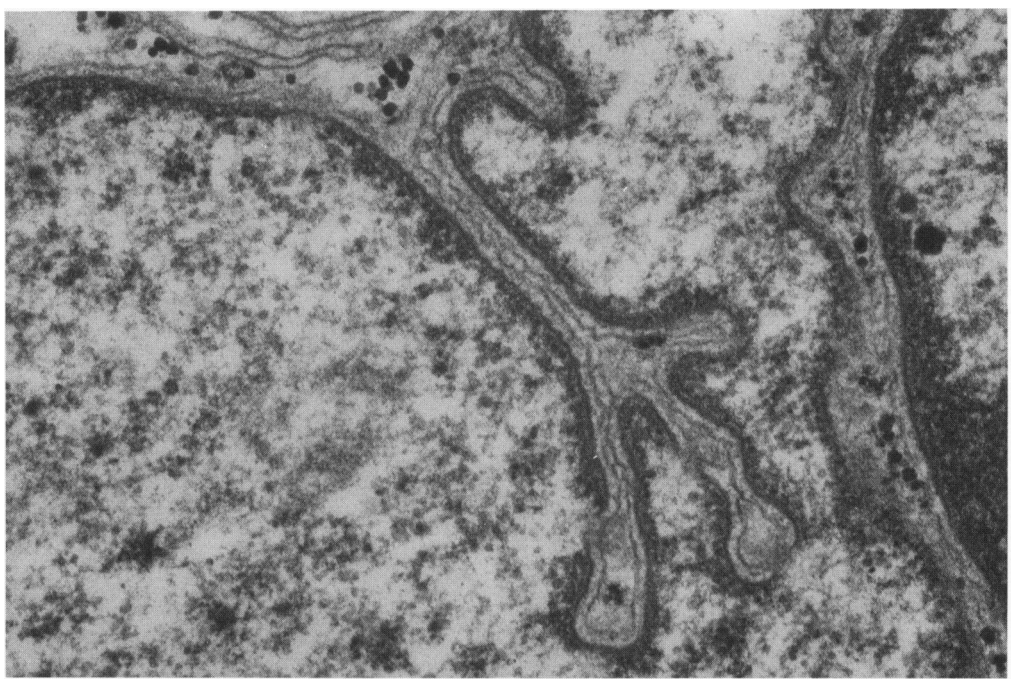

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Fig 16—Nucleus of cardiac muscle cell, from same patient as in Figure 14, contains two types of intranuclear tubules. Intranuclear tubules of the first type are similar to those shown in Figure 15, originate from invagination of the inner nuclear membrane (*white arrowheads*) and are associated with chromatin; tubules of the second type (*black arrowheads*) are irregular in contour and are not associated with chromatin (× 40,000).
Fig 17—Lateral view of convoluted folds involving both inner and outer nuclear membranes. As shown in this illustration, both membranes are clearly distinguishable, even in very narrow folds. This differentiates between tangentially cut folds and the tubular invaginations of inner nuclear membranes shown in Figures 15 and 16. Same patient as in Figures 14 and 16 (× 63,000).

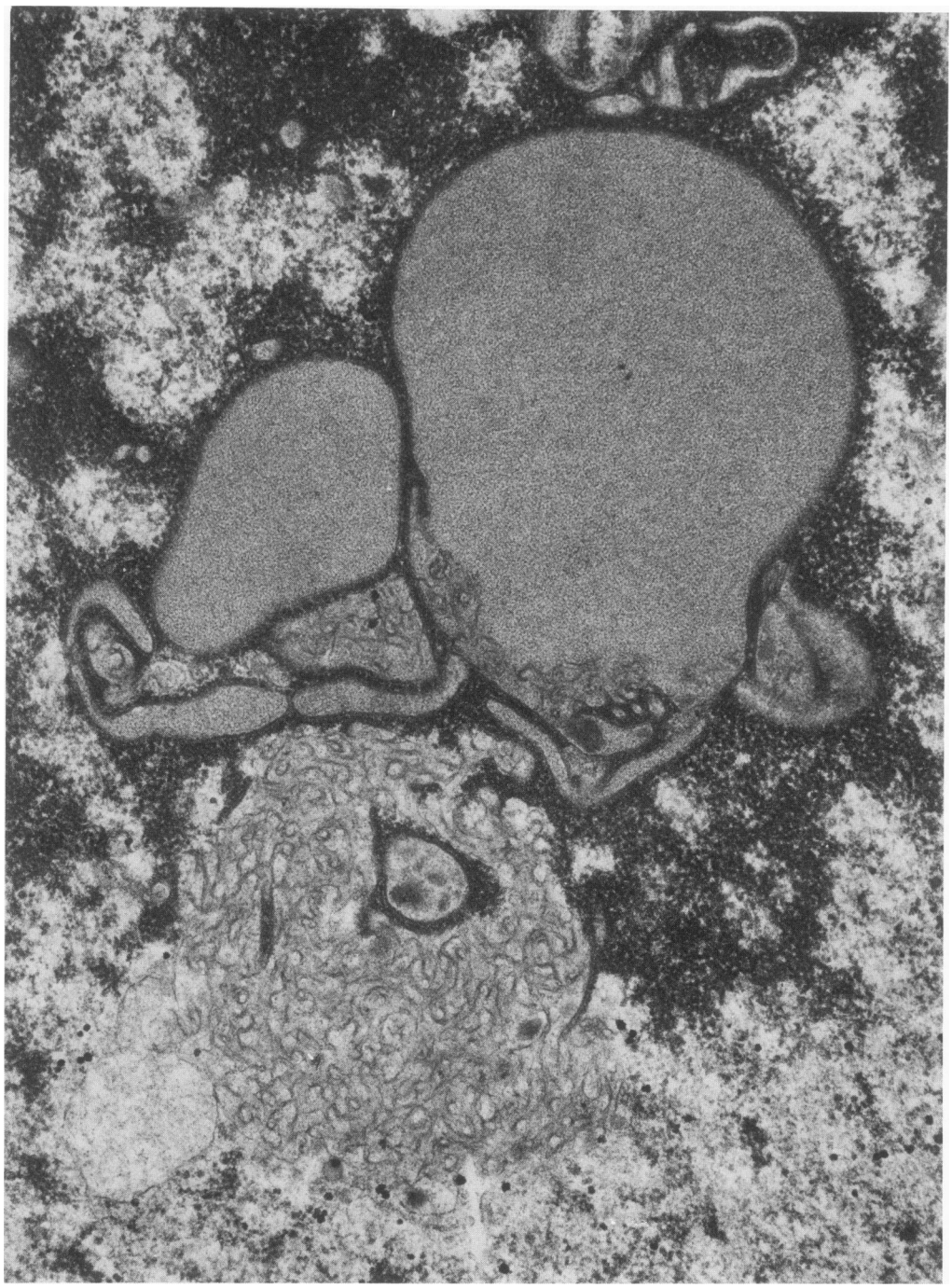
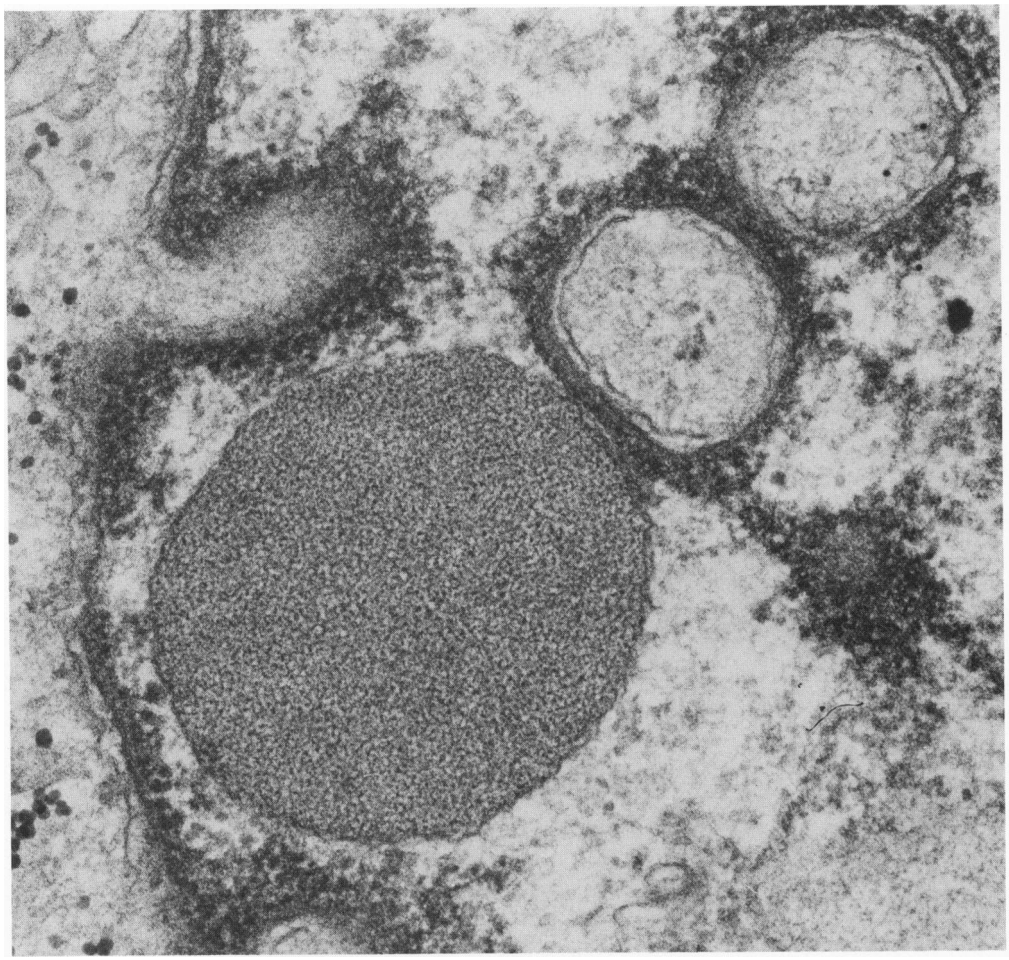


Fig 18—Tangentially cut section through nucleus of cardiac muscle cell shows two types of intranuclear tubules. Tubules of the first type (*top and center*) are formed by inner nuclear membranes which are associated with marginated chromatin; some of these tubules (*top*) have clear lumina, while others (*center*) are filled with finely granular material similar to that in the nuclear inclusions. The latter also are limited by inner nuclear membranes associated with marginated chromatin. Tubules of the second type are highly convoluted and form a tangled mass at the bottom of the picture. Same patient as in Figures 14, 16 and 17 (× 34,500).



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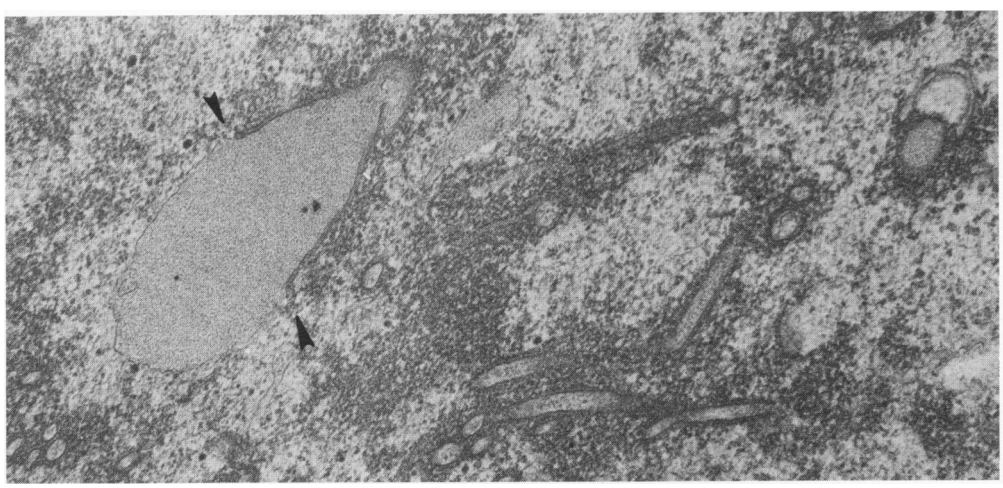

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Fig 19—High magnification view of nuclear inclusion in cardiac muscle cell from same patient as in Figure 12. The inclusion contains finely granular, electron-dense material and is limited by inner nuclear membrane which is not associated with marginated chromatin (× 72,000). **Fig 20**—Nuclear inclusion and intranuclear tubules are present in cardiac muscle cell from same patient as in Figure 8. The nuclear inclusion is limited by inner nuclear membrane; the association of this membrane with chromatin is clearly shown to terminate abruptly (*arrowheads*). Some intranuclear tubules contain material similar to that within the inclusion (× 30,000).

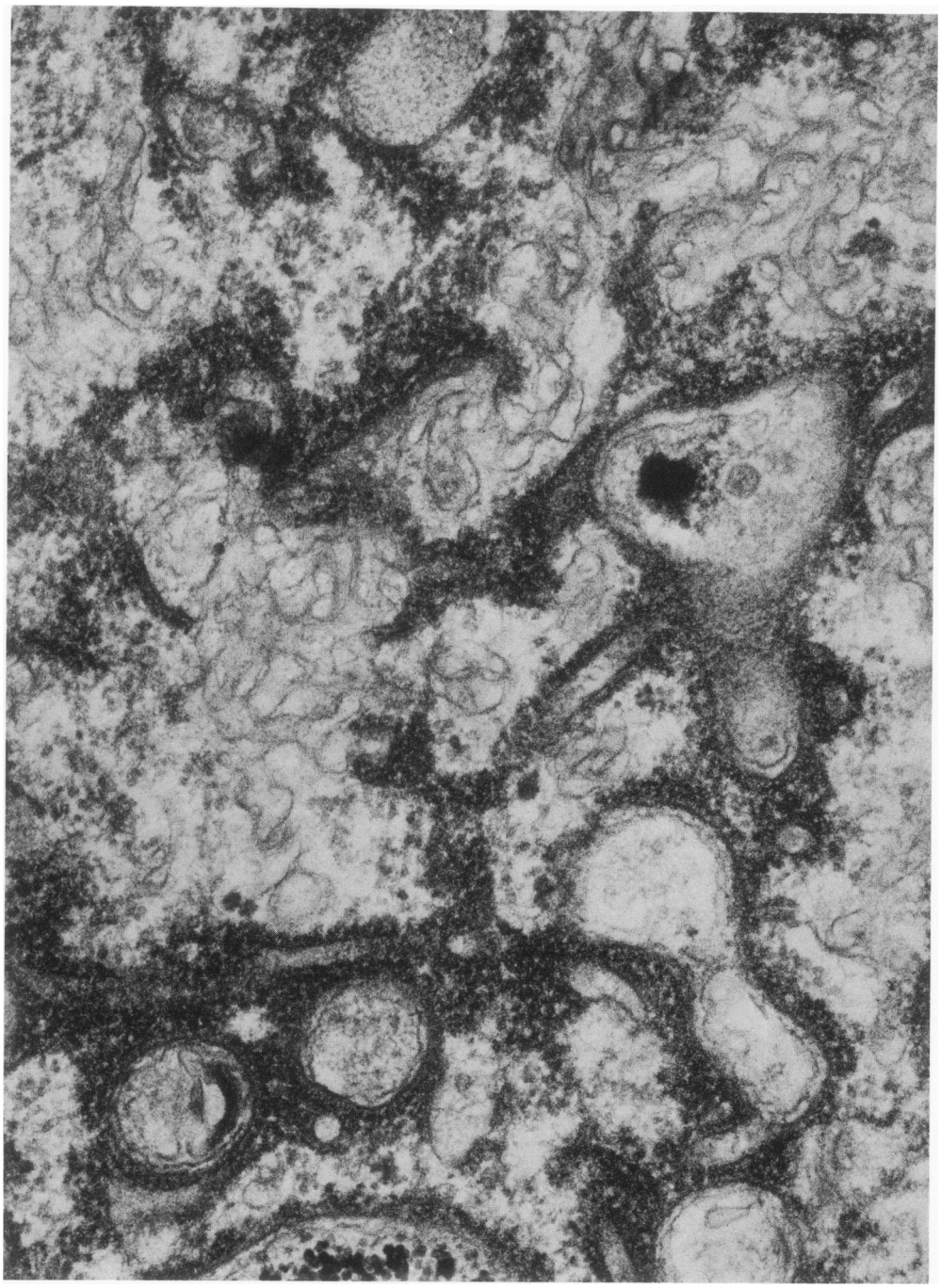

Fig 21—High magnification view of convoluted, coiled intranuclear tubules not associated with chromatin. Compare with Figure 18. A few, larger tubules associated with chromatin also are present. Cardiac muscle cell from same patient as in Figures 12 and 19 (× 63,000).