The Effect of Verapamil on the Calcium Paradox

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Reperfusion of isolated rat hearts with calcium-containing medium after a short period of calcium-free perfusion results in irreversible cell damage (calcium paradox). Experiments were undertaken to determine whether the slow-channel calcium-antagonist drug verapamil protects calcium-deprived rat heart muscle against the consequences of readmitting calcium. Cell damage was quantitated in terms of creatine kinase (CK) release, depletion of endogenous creatine phosphate (CP) and adenosine triphosphate (ATP) stores, development of contracture as measured by longitudinal shortening of the left ventricle, and ultrastructural damage. Verapamil (1 mg/l) did not reduce the initial rate of CK release during reperfusion with calcium but reduced the initial rate at which myocardial CP and ATP stores were depleted and decreased the shortening of the longitudinal axis of the left ventricle. After 30 seconds of reperfusion the mean sarcomere length was significantly greater in the verapamil-treated hearts. These results can be interpreted to mean that inhibition of calcium influx via the slow channels does not protect heart muscle against the deleterious effects of readmitting calcium after a period of calcium-free perfusion. (Am J Pathol 1980, 98:769-790)

WHEN ISOLATED HEARTS are perfused with a calcium-free medium, mechanical activity ceases [1] and electrical activity continues.[2] Reperfusion with a calcium-containing medium after only three minutes of calcium-free perfusion results in the calcium paradox.[3] This phenomenon is characterized by an influx of calcium into the cells,[4,5] the rapid onset of myocardial contracture,[6,7] exhaustion of tissue high-energy phosphates,[8,9] massive release of cell constituents,[3,10] and extensive ultrastructural damage.[11-13]

It has been postulated that during calcium-free perfusion the permeability of the sarcolemma to calcium changes in such a way that reperfusion in the presence of calcium results in intracellular calcium overload.[4,11,13] In an attempt to reduce the damaging effect of sequential perfusion with calcium-free and calcium-containing medium, the slow-channel calcium-antagonist drug verapamil [14-16] was added to the calcium-free medium and the reperfusion medium. We argued that if the enhanced entry of calcium that occurs during the calcium paradox involved the entry of these ions through the slow calcium channels, then drugs that

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Supported in part by the Medical Research Council of Great Britain, the Wellcome Trust, and Knoll AG (Ludwigshafen, Germany).

Accepted for publication October 5, 1979.

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0002-9440/80/0310-0769$01.00

inhibit slow calcium channel transport could be protective. Cell damage was quantitated in terms of enzyme leakage, depletion of high-energy phosphates, development of myocardial contracture as measured by longitudinal shortening of the left ventricle, and ultrastructural damage.

Materials and Methods

Perfusion Technique

Male Wistar rats of 200–250 g were anesthetized with diethyl ether. The rats were heparinized and the hearts quickly removed and subsequently perfused at 37 C by the Langendorff technique [17] at a constant pressure of 10.0 kP_a(75 mm Hg). Four perfusion fluid reservoirs were connected to the aortic cannula by means of a specially designed stop-cock allowing alternate use of the reservoirs. The standard perfusion medium had the following composition (mmol/l): NaCl, 124; KCl, 4.7; $CaCl_2$, 1.3; $MgCl_2$, 1.0; $NaHCO_3$, 24.0; Na_2HPO_4, 0.5; glucose, 11.0. During calcium-free perfusion, calcium was omitted from the standard medium; no correction was made for the small change of osmolarity. Great care was taken to ensure that this solution and all its associated glassware were free of calcium.[6] The mediums were equilibrated with 95% O_2 − 5% CO_2 to provide a P_{O_2} of >80.0 kP_a(600 mm Hg). After equilibration the pH of the mediums was 7.40 ± 0.05. The isotonic contractions of the heart were recorded as described by Meijler et al [18] and as used in our earlier studies of the calcium paradox.[3,6,7]

After a 15-minute stabilization period, during which the hearts were perfused with standard medium, the perfusion was changed to the calcium-free medium. After 4 minutes of calcium-free perfusion, the hearts were reperfused with the calcium-containing medium. When required, 1 mg/l racemate verapamil (Knoll AG, Ludwigshafen, F.R.G.) was added to the calcium-free medium and the reperfusion medium. This concentration of verapamil was used because it has been shown to protect the hypoxic heart [19] and because it is in excess of that required to block the slow calcium channels in heart muscle.[20]

Analytical Procedures

Samples of the effluent medium were collected and analyzed for creatine kinase (CK) (adenosine triphosphate (ATP): creatine phosphotransferase E.C.2.7.3.2.) activity. CK activity was assayed by the method of Oliver,[21] modified by Rosalki,[22] with the use of a Vitatron Automatic Kinetic Enzyme System (AKES) at 25 C. Enzyme activity was expressed in IU released/min/g dry heart tissue (±SEM).

The perfusion of hearts on which creatine phosphate (CP) and adenosine triphosphate determinations were to be made was terminated by freezing the hearts between large aluminum tongs, precooled in liquid nitrogen.[23] The frozen tissue was assayed for CP and ATP as previously described.[8] Myocardial CP and ATP content was expressed in μmol/g dry weight (±SD).

Electron Microscopy

Control hearts were perfused for 15 minutes with standard medium and then perfusion-fixed with 4% glutaraldehyde prepared in 0.2 mol/l sodium cacodylate buffer (pH 7.3). Other hearts were perfused with calcium-free medium for 4 minutes, reperfused with calcium-containing medium for either 30 seconds or 30 minutes, with or without verapamil, and subsequently perfusion-fixed. After 10 minutes of glutaraldehyde perfusion, the hearts were removed from the Langendorff apparatus, and biopsy specimens of the left ventricle free wall were excised and cut into 1-cu-mm cubes. These were immersion-fixed in the glutaraldehyde solution for 2 hours, then postfixed for 2 hours in 1% OsO_4. Samples were

then stained *en bloc* with saturated aqueous uranyl acetate for 30 minutes, dehydrated with the use of ethyl alcohol, and embedded in epoxy resin. Blocks were sectioned on an LKB Ultratome III with a diamond knife, and silver sections were examined in a Philips 301 electron microscope.

Five hearts were processed for each series (control, calcium paradox after 30 seconds of reperfusion, and calcium paradox after 30 seconds of reperfusion with verapamil added). Four blocks were sectioned at two levels (orientated longitudinally) for each heart, and five micrographs were taken for each level. Fiber orientation for each block was established with the use of thick sections for light microscopy. However, in some areas of gross damage and contracture, fiber orientation was difficult to determine. Because the likelihood of producing tangentially orientated sections was the same in the verapamil-treated and untreated hearts, errors introduced by tangential sections would be distributed equally between the different series of experiments. Micrographs were recorded with a regular pattern of every second specimen grid square overlying the section. Before recording each micrograph, the necessary procedure for hysteresis elimination was carried out. The micrographs were recorded at the same magnification setting, and each cassette of micrographs included a diffraction grating to check for any magnification variation in the series. A reference grid was superimposed on each print to check for distortion of the print during printing and drying. A regular array of the grid squares formed on the image was used to obtain an unbiased area for measurement. Sarcomeres lying within the pattern of squares in the array were measured and scored into ranks of 0.2 μ categories from 0 to 2.2 μ. Electron micrographs were examined blind. The data was analyzed statistically.

Statistical Analysis

For electron microscopy, the significance of any difference in sarcomere length in three groups was analyzed with the use of the Komolgorov–Smirnov two-sample test. The three groups were: 1) control versus calcium paradox with verapamil; 2) control versus calcium paradox without verapamil; 3) calcium paradox with verapamil versus calcium paradox without verapamil.

The Student t test was used in Table 1 and Text-figures 2 and 3, taking $P = 0.05$ as the limit of significance.

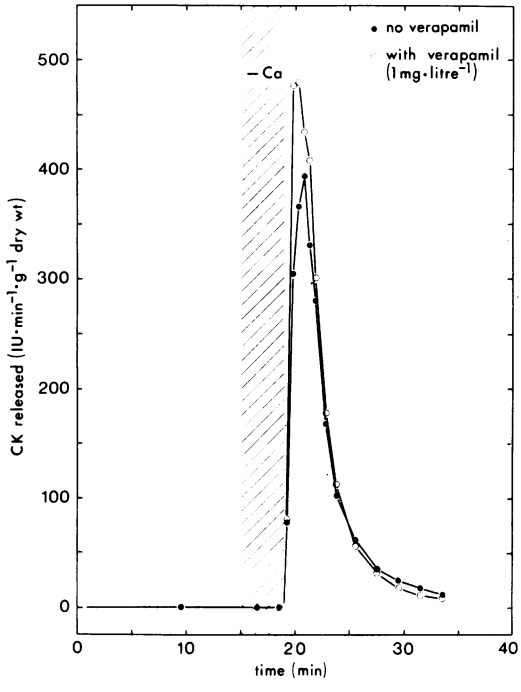
Results

Effect of Verapamil on the Calcium-Induced Release of CK

During the stabilization (0–15 minutes) and subsequent calcium-free period (15–19 minutes) the hearts released negligible amounts of CK into the coronary effluent. Reintroducing calcium into the perfusion medium resulted in an immediate and massive release of CK (Text-figure 1). When verapamil was added to the calcium-free medium and to the reperfusion medium, the initial rate of CK release during reperfusion with calcium-containing medium was not significantly different from that in the experiments with untreated hearts (Text-figure 1, Table 1).

Effect of Verapamil on the Calcium-Induced Depletion of Tissue CP and ATP Stores

At the end of the stabilization period myocardial CP and ATP stores amounted to 29.6 ± 2.1 and 18.8 ± 0.8 μmol/g dry weight, respectively (Text-figure 2). Subsequent calcium-free perfusion for 4 minutes resulted



TEXT-FIGURE 1—Effect of verapamil (1 mg/l) on the release of CK (IU/min/g dry weight) from isolated rat heart, induced by reperfusion with calcium after a calcium-free period of 4 minutes (*shaded bar*). When verapamil was present, it was added to the calcium-free medium and the reperfusion medium. Each point represents the mean of four separate determinations. The details of these points are included in Table 1.

in a slight increase in the endogenous stores of CP and ATP, irrespective of whether verapamil was present. During the first minute of reperfusion with calcium, myocardial CP fell from 35.6 ± 2.9 to 4.6 ± 3.2 μmol/g dry weight in the absence of verapamil, and from 33.1 ± 2.6 to 15.0 ± 2.2 μmol/g dry weight in the presence of verapamil ($P < 0.001$). In the same period myocardial ATP fell from 20.8 ± 1.4 to 6.3 ± 2.7 μmol/g dry weight in the absence of verapamil, and from 20.6 ± 1.5 to 13.2 ± 1.2 μmol/g dry weight in the presence of verapamil ($P < 0.001$). Text-figure 2 shows that although the rate of consumption of the tissue stores of ATP and CP during reperfusion was slower in the verapamil-treated than in the untreated hearts, after 4 minutes of reperfusion with calcium-containing solution there was no difference between the ATP and CP content of treated and untreated hearts.

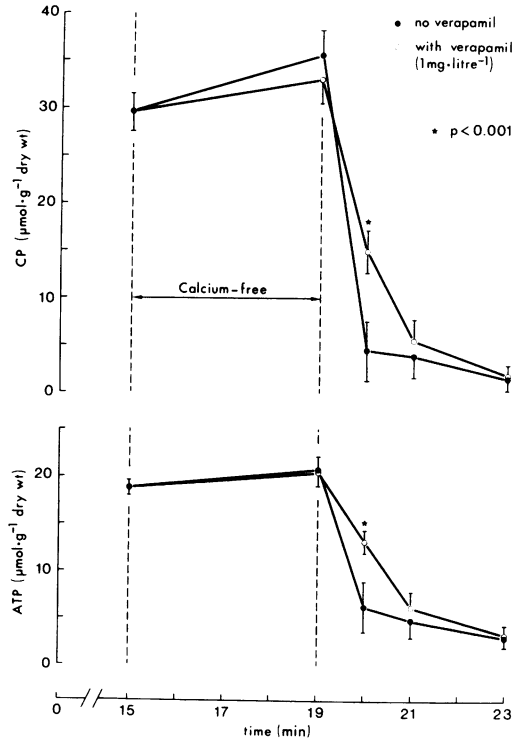
Effect of Verapamil on the Calcium-Induced Contracture

During the stabilization period isotonic contractions were recorded as changes in the length of the longitudinal axis of the left ventricle, as previously reported.[6] Omitting calcium from the medium caused rapid arrest in diastole. Verapamil had no effect on the degree of relaxation achieved during diastole. Reperfusion with calcium in the absence of verapamil re-

Table 1—Effect of 1 mg/l Verapamil on Perfusion Flow and Release of CK During Successive Perfusion With Calcium-Free and Calcium-Containing Medium

Perfusion period (min)	Perfusion flow (ml/min)			CK release (IU/min/g dry weight)		
	No verapamil	Verapamil	*P*	No verapamil	Verapamil	*P*
Control perfusion (0–15)						
9–10	7.9 ± 0.9			0		
Ca^{2+}-free perfusion (15–19)						
16–17	13.5 ± 2.6	12.4 ± 2.2	NS	0	0.8 ± 0.1	
18–19	13.1 ± 2.6	11.7 ± 2.3	NS	0	1.0 ± 0.1	
Reperfusion with Ca^{2+} (19–35)						
19 –19.5	5.4 ± 0.9	10.7 ± 1.2	<0.001	78 ± 15	78 ± 24	NS
19.5–20	2.2 ± 0.7	5.7 ± 0.2	<0.001	304 ± 15	476 ± 69	NS
20 –20.5	2.5 ± 0.9	4.2 ± 0.2	<0.02	366 ± 70	478 ± 54	NS
20.5–21	3.1 ± 1.0	4.3 ± 0.2	NS	393 ± 14	432 ± 36	NS
21 –21.5	3.7 ± 0.8	4.7 ± 0.1	NS	330 ± 56	407 ± 16	NS
21.5–22	3.8 ± 0.9	4.8 ± 0.3	NS	281 ± 35	300 ± 9	NS
22 –22.5	4.0 ± 0.7	4.7 ± 0.4	NS	223 ± 17	202 ± 9	NS
22.5–23	4.0 ± 0.8	4.9 ± 0.3	NS	167 ± 13	178 ± 9	NS
23 –23.5	4.2 ± 0.9	4.9 ± 0.3	NS	139 ± 6	148 ± 7	NS
23.5–24	4.2 ± 1.0	4.9 ± 0.3	NS	102 ± 4	112 ± 8	NS
25 –26	4.7 ± 1.1	5.1 ± 0.3	NS	62 ± 6	58 ± 5	NS
27 –28	4.8 ± 1.2	5.2 ± 0.4	NS	35 ± 5	32 ± 7	NS
29 –30	5.0 ± 1.1	5.3 ± 0.3	NS	24 ± 3	18 ± 2	NS
31 –32	5.1 ± 1.2	5.3 ± 0.3	NS	18 ± 1	11 ± 1	<0.001
33 –34	5.1 ± 1.3	5.4 ± 0.3	NS	12 ± 1	9 ± 1	<0.05

Results are expressed as mean ± SD (perfusion flow) or as mean ± SEM (CK release) of four perfusion experiments. During the different perfusion periods the effluent medium was collected and analyzed for CK activity. When verapamil was present, it was added to the calcium-free medium and the reperfusion medium. Tests of significance relate to significance of change in perfusion flow and CK release caused by the presence of verapamil. NS = not significant.



TEXT-FIGURE 2—Effect of verapamil (1 mg/l) on the depletion of myocardial CP and ATP stores, induced by reperfusion with calcium after a calcium-free period of 4 minutes. When verapamil was present, it was added to the calcium-free medium and the reperfusion medium. Values are given as mean ± SD (n = 4). Tests of significance relate to the significance of the difference between CP and ATP levels in hearts perfused in the presence and absence of verapamil. In certain cases, for the purpose of clarity, only one SD bar has been given.

sulted in the development of an irreversible contracture, as measured by longitudinal shortening of the left ventricle. Text-figure 3 shows that in the presence of verapamil this contracture was significantly reduced.

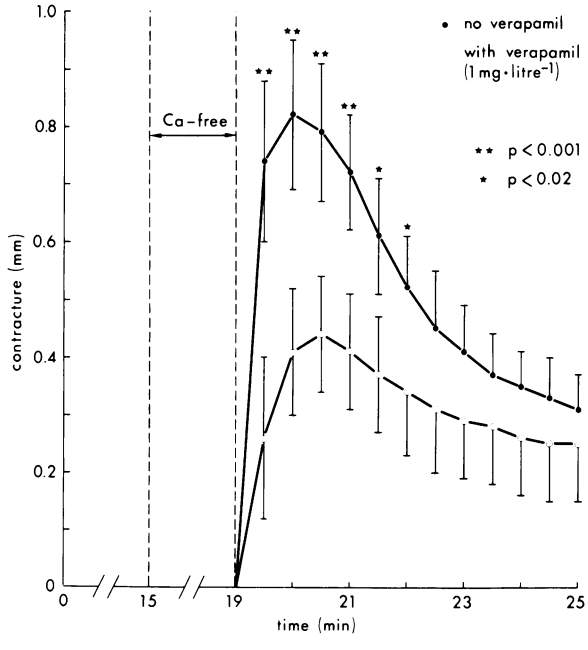
Ultrastructural Results

At the end of the 15-minute stabilization period of perfusion with standard medium, the ultrastructure of the hearts was normal (Figure 1). After a subsequent calcium-free period of 4 minutes, followed by 30 seconds of reperfusion with calcium-containing medium, many fields in both the verapamil-treated (Figures 2 and 3) and untreated (Figures 4 and 5) hearts appeared to show relaxed myofibrils. However, when the mean sarcomere length of each series (control, calcium paradox, and calcium paradox with verapamil) was measured, a difference was found. The untreated series had a mean sarcomere length of 1.08 μ, whereas the verapamil-treated series had a mean sarcomere length of 1.33 μ (Table 2). This difference was significant ($P < 0.001$). Text-figure 4 shows the distribution of sarcomere lengths for each series. After this short period of reperfusion the mitochondria did not contain any electron-dense granules. After 30 minutes of

reperfusion with calcium-containing medium the hearts all showed evidence of extensive damage, irrespective of whether verapamil had been added (Figures 6 and 7). Every field showed gross tissue damage and severe contracture, and no quantitative analysis was undertaken. In contrast to the condition described after 30 seconds of reperfusion, after 30 minutes of reperfusion electron-dense granules of the calcium type (Figure 8) were present in the mitochondria, irrespective of whether verapamil had been included in the perfusion fluid.

Discussion

It is generally agreed that during calcium-free perfusion the cell membrane changes in such a way that reperfusion in the presence of calcium

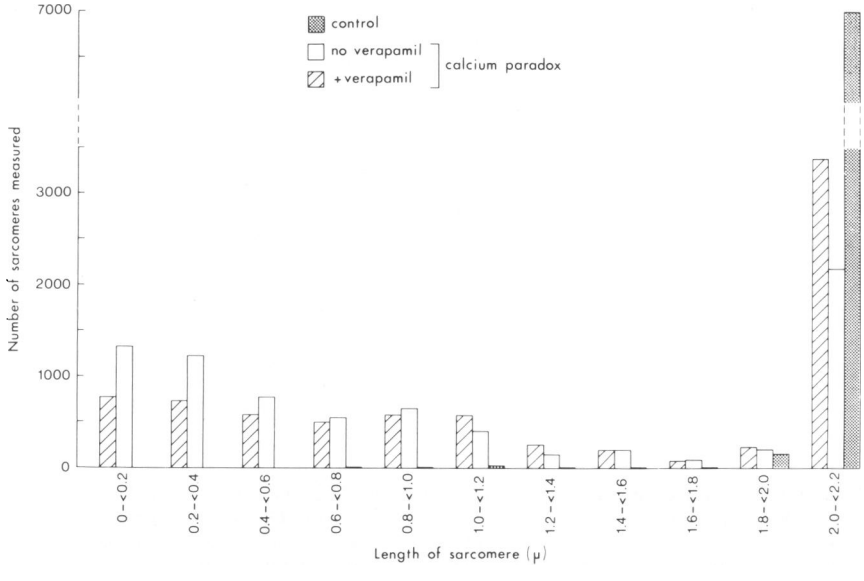


TEXT-FIGURE 3—Effect of verapamil (1 mg/l) on the development of myocardial contracture as measured by longitudinal shortening of the left ventricle, induced by reperfusion with calcium after a calcium-free period of 4 minutes. Spontaneously beating rat hearts went into diastolic arrest as soon as calcium was omitted from the medium. Reperfusion with calcium resulted in the development of irreversible contracture. Contracture is expressed as shortening of the length (mm) of the longitudinal axis of the left ventricle. Values are given as mean ± SD (n = 4). Tests of significance relate to the significance of the difference between contracture of hearts perfused in the presence and absence of verapamil. In certain cases, for purposes of clarity, only one SD bar has been given.

Table 2—Effect of 1 mg/l Verapamil on Mean Sarcomere Length in Calcium Paradox Hearts

Perfusion sequence	*n*	Mean sarcomere length (μ)	SEM
15 min control perfusion	7292	2.18	0.001
15 min control perfusion; 4 min Ca^{2+}-free perfusion + verapamil; 30 sec reperfusion with Ca^{2+} + verapamil	7671	1.33	0.009
15 min control perfusion; 4 min Ca^{2+}-free perfusion; 30 sec reperfusion with Ca^{2+}	7991	1.08	0.009

leads to an increased entry of calcium [4,5] and the formation of intramitochondrial electron-dense deposits.[4,10,11,13] Although the exact mechanisms leading to the calcium paradox damage remain to be elucidated, mitochondria, with their ability to accumulate massive amounts of calcium, may play an important role in the origin of the phenomenon. The mitochondrial accumulation of calcium, together with phosphate ions, can be supported by ATP or electron transport. During electron-transport–supported calcium accumulation mitochondria are not able to phosphorylate ADP.[24] This process may account for the rapid CP and ATP depletion during the calcium paradox. The finding that the conditions for



TEXT-FIGURE 4—Distribution of sarcomere lengths (μ) in three series of hearts. The control series was perfused for 15 minutes with standard medium. The calcium paradox series was perfused for 15 minutes with standard medium, then for 4 minutes with calcium-free medium, and then reperfused for 30 seconds with calcium-containing medium. In the calcium paradox + verapamil series 1 mg/l verapamil was added to the calcium-free medium and the reperfusion medium.

the occurrence of the calcium paradox (availability of energy either in the form of ATP or produced by electron transport) are the same as for mitochondrial calcium accumulation lends support to the hypothesis that mitochondria play a crucial role in the origin of the calcium paradox.[25] The present studies were undertaken to establish whether a drug that inhibits calcium transport through the slow channels would prevent the occurrence of the calcium paradox.

Depletion of myocardial high-energy phosphate stores is another factor leading to an increased permeability of the cell membrane to calcium, as demonstrated in hypoxic [19] and ischemic [26] heart muscle. The biochemical and ultrastructural changes induced by reoxygenating anoxic myocardium [27-29] and by reperfusing ischemic muscle [29-31] are apparently identical to those noted in hearts during the calcium paradox and have also been attributed to energy-dependent calcium fluxes.[32] Comparison of the enzyme release characteristics of calcium-deprived hearts upon reperfusion with calcium and of energy-deprived hearts upon readmission of oxygen,[25,29] however, indicate that the membrane damage induced by calcium-free perfusion is different from and more severe than that caused by energy depletion. Recently, the effect of removal of calcium from the extracellular fluid on the structure of the sarcolemma has been described in detail.[33,34]

The present results show that verapamil provides calcium-deprived hearts only limited protection against the consequences of reperfusion with calcium. The most conspicuous effect of verapamil on the calcium paradox was a reduced rate of depletion of the myocardial CP and ATP stores. This energy-sparing effect of verapamil was only perceptible during the first few minutes of reperfusion with calcium-containing medium. The reduced rate of ATP depletion may account for the reduction in magnitude of myocardial contracture as measured by longitudinal shortening of the left ventricle (Text-figure 3) and for the less severe decrease of mean sarcomere length after 30 seconds of reperfusion in the presence of verapamil (Table 2), because ATP depletion is a determining factor in the development of irreversible myocardial contracture.[35,36] In addition, interventions that conserve cellular ATP supplies have been shown to delay the onset of contracture.[37] Not all cells were affected by the readmission of calcium (Figures 2 and 3), indicating that the response is heterogeneous. A similar situation has been described for the ischemic heart.[38] After 30 minutes of reperfusion all cells were heavily damaged and most remnants of the contractile apparatus were in a contracted state, irrespective of whether verapamil was present (Figures 6 and 7). Hence, the reversal of longitudinal shortening that began within two minutes of reper-

fusion (Text-figure 3) was possibly artifactual and caused by the grossly distorted architecture of the heart rather than a relaxation of the myofibrils.[3,6,7,11]

The deposition of electron-dense granules of the calcium type in mitochondria appears to be a relatively late event in hearts during the calcium paradox. In the present study granules were not present after 30 seconds of reperfusion. However, granules have been shown to be present after 10 minutes of reperfusion with calcium-containing medium.[11] The fact that there was no difference in the distribution of dense granules after 30 minutes of reperfusion with calcium-containing solution in the verapamil-treated and untreated hearts suggests that verapamil had not prevented an excessive entry of calcium upon reintroduction of calcium in the perfusion medium. The granules were composed of smaller dense particles (Figure 8) and had the same appearance as those seen in calcium-phosphate–loaded mitochondria.[39] Electron-lucent cores, as have been observed in some of the mitochondrial granules after calcium phosphate accumulation,[39,40] were not seen. In the present study no attempt was made to monitor the rate of calcium influx during reperfusion. The late finding of mitochondrial calcium dense bodies in both the verapamil-treated and untreated hearts does not imply either that an enhanced influx of calcium did not occur from the start, long before granules were visible, or that the rate of calcium influx was similar in both groups.

Verapamil was unable to protect the cellular integrity against the consequences of the readmission of calcium. On the contrary, the initial rate of CK release from verapamil-treated hearts was even higher than from untreated hearts, although the difference was not significant. This difference is probably caused by the higher coronary flow in the verapamil-treated hearts. The increase in coronary flow may be due to the less pronounced contracture (Text-figure 3) or the vasodilator action of the drug,[14] giving rise to a more rapid removal of CK from the damaged cells.

Our data on the energy-sparing effect of verapamil shows a qualitative resemblance to the results obtained by Nayler et al [19] in hypoxic heart muscle. This resemblance, however, is superficial because in the hypoxic hearts the tissue levels of ATP and CP fall because of impaired metabolism, and calcium influx is enhanced as a secondary effect. This situation contrasts with that described for hearts during the calcium paradox,[8] where the endogenous stores of ATP and CP are well maintained during the period of calcium-free perfusion and hence are normal at the time when calcium is reintroduced to trigger the calcium paradox. Therefore, reintroduction of calcium in the medium will cause an enhanced calcium influx and, as a secondary effect, ATP and CP depletion. Although vera-

pamil failed to prevent the hypoxic muscle from gaining calcium, it reduced the rate at which myocardial CP and ATP stores were depleted [19] and in so doing possibly ensured that sufficient ATP remained available to maintain intracellular calcium homeostasis. Similar results were obtained with methylprednisolone sodium succinate.[41] In addition, the presence of methylprednisolone sodium succinate during the period of hypoxic perfusion facilitated the recovery of mechanical activity upon reoxygenation.[42]

In conclusion, our results show that verapamil does not prevent the calcium paradox. Therefore, it must be assumed that calcium flux through the slow channels is evidently not the only or primary source of calcium for the paradox. However, verapamil protects heart muscle against some of the deleterious effects of readmitting calcium. Verapamil has an energy-sparing effect and reduces the decrease of the mean sarcomere length upon reperfusion of calcium-deprived hearts with calcium-containing medium. The basis of this energy-sparing effect is unknown, but there are several possibilities that should be considered. Because of the drug's negative inotropism, which could be expressed during the first few seconds of the calcium paradox and hence at the time when some active tension development is still occurring in response to membrane depolarization, the rate of depletion of the endogenous stores of ATP and CP could have been slowed. Under these conditions more ATP and CP would remain available for the maintenance of intracellular calcium homeostasis at the time when calcium was readmitted to the extracellular space. Another possibility is that by inhibiting slow-channel transport early during the reperfusion process, verapamil could have reduced the rate at which mitochondria ceased producing ATP because of calcium overload. Under these conditions more ATP and CP would remain available for the maintenance of calcium homeostasis. At the moment we cannot distinguish between these two possibilities. However, since calcium-free cardioplegic solutions are widely used to arrest the heart during open-heart surgery,[43–45] additional studies are needed to obtain more insight into the mechanisms leading to the calcium paradox and to the possible use of drugs to prevent its occurrence.

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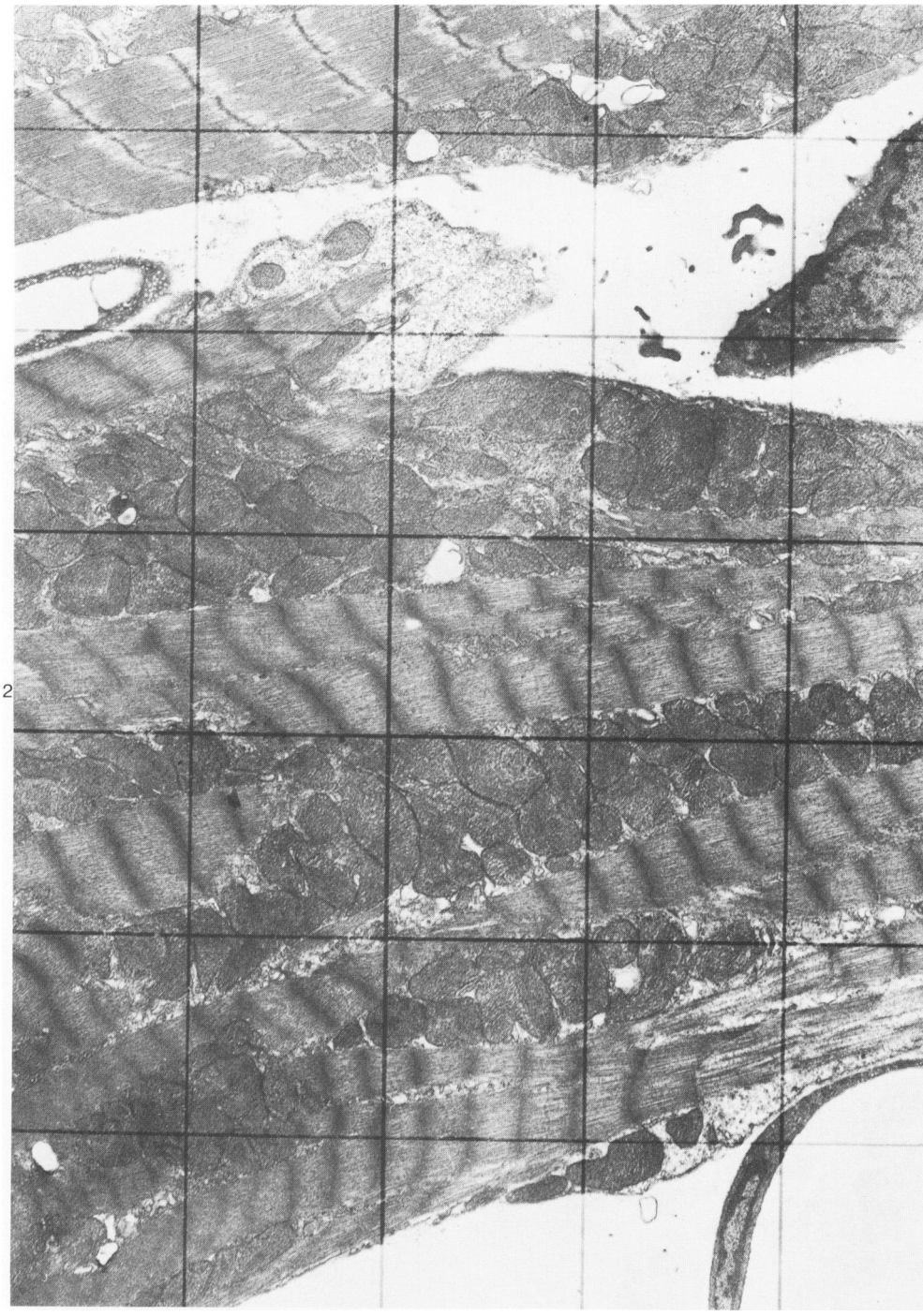
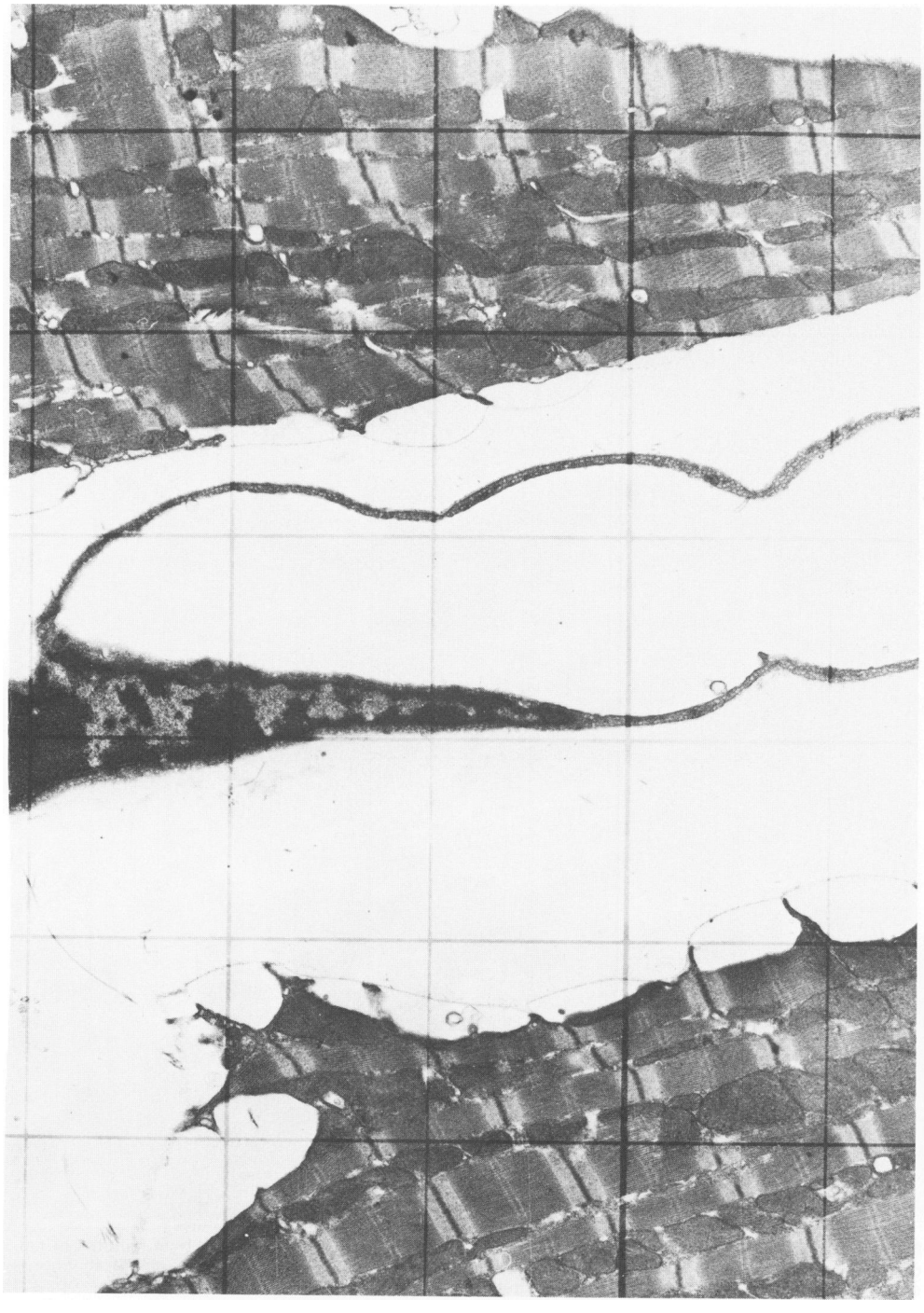
Acknowledgments

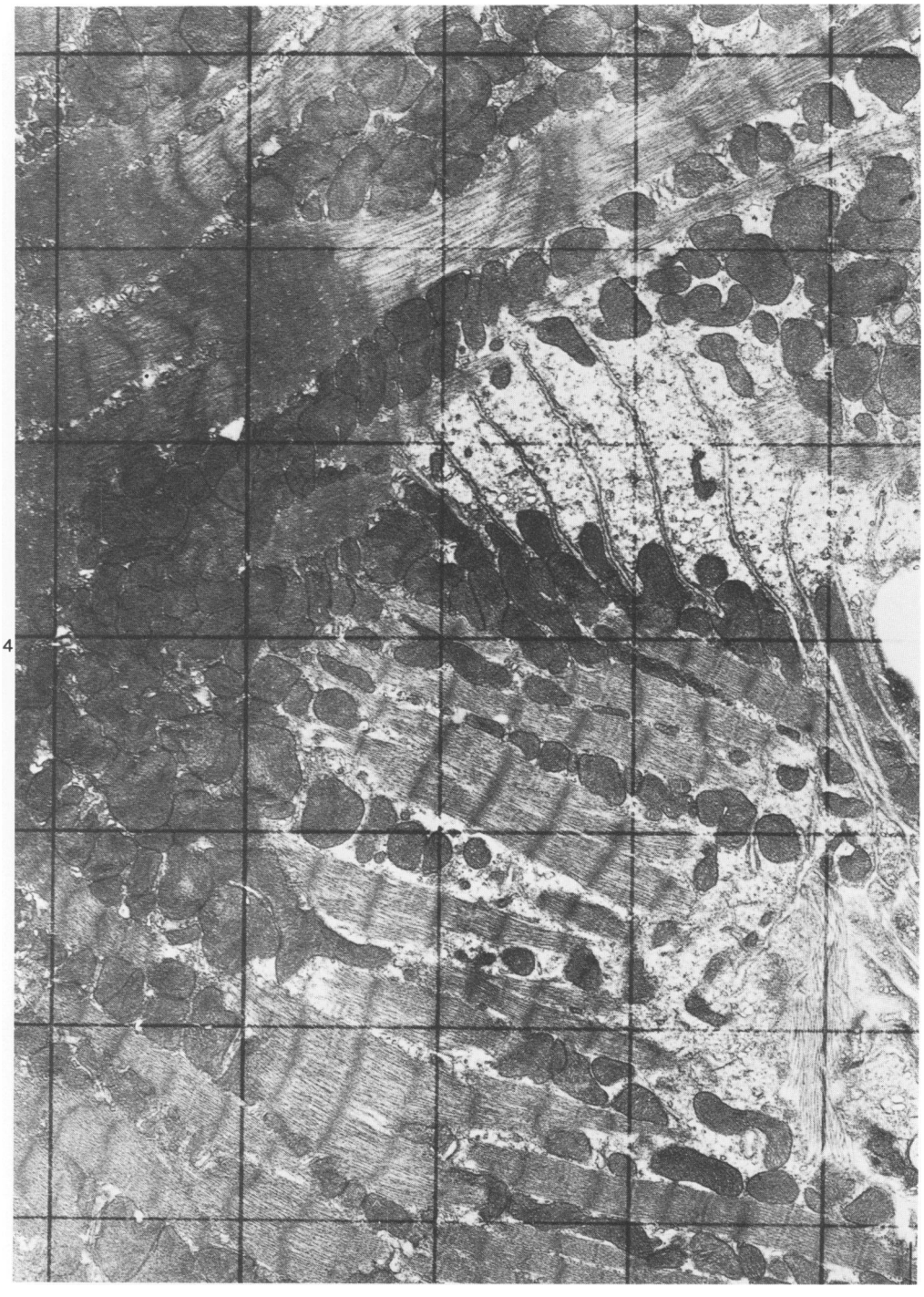
Dr. G. Pfennigsdorf provided support, and Dr. A. H. J. Maas provided interest and encouragement.

Figure 1—Control heart perfused for 15 minutes with standard medium. The heart has the appearance of normal myocardium. Myofibrils are in register and mitochondria have an electron-opaque matrix. (Perfusion fixation; uranyl acetate, ×7500)

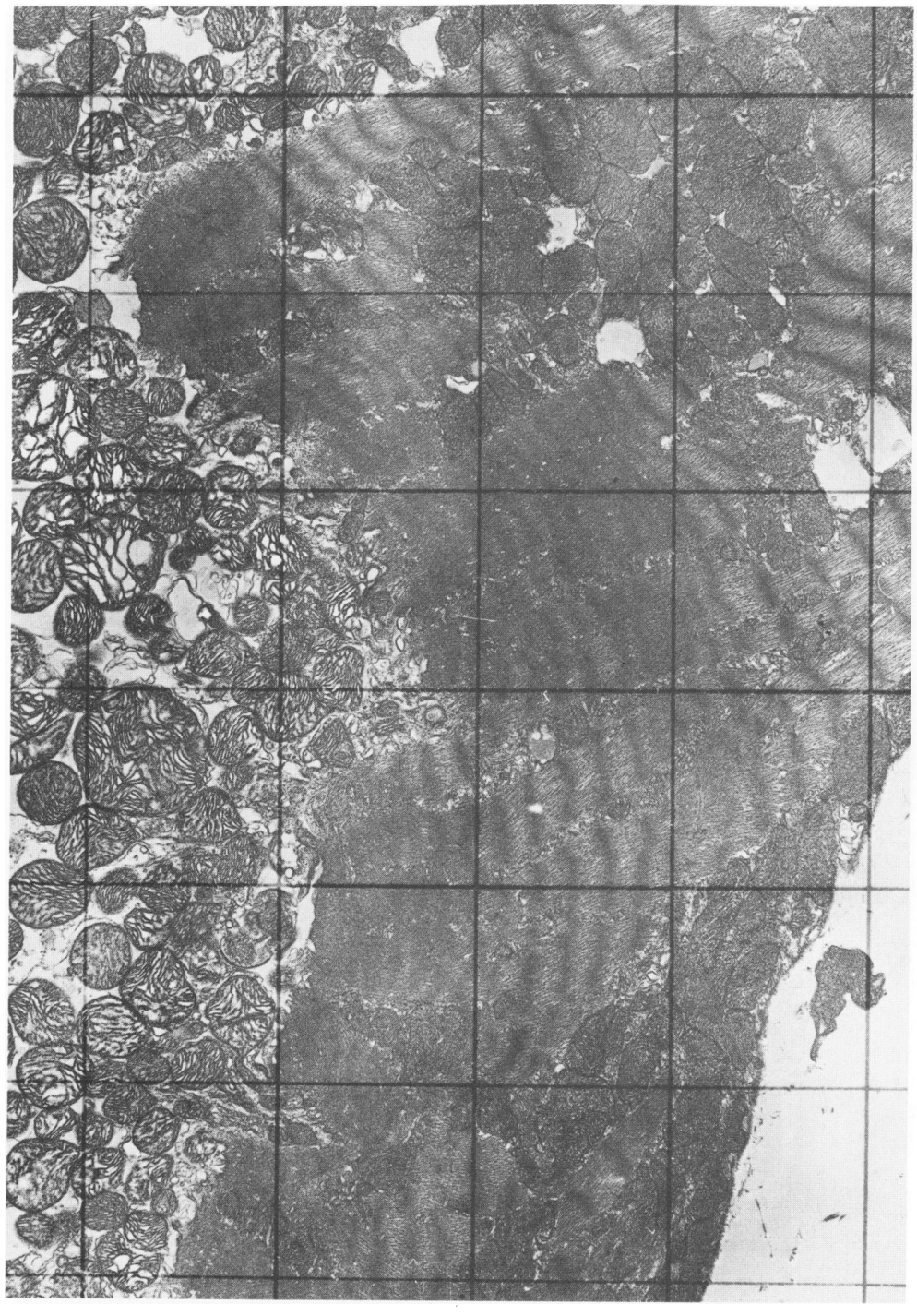


Figures 2 and 3.—Heart perfused for 15 minutes with standard medium, followed by 4 minutes of calcium-free perfusion and 30 seconds of reperfusion with calcium-containing medium. The calcium-free medium and the reperfusion medium contained 1 mg/l verapamil. The myofibrils in Figure 2 are contracted, whereas the myofibrils in Figure 3 are relaxed, demonstrating the heterogeneous manner in which heart muscle responds to the readmission of calcium. The cells of Figure 3 show separation of the basement membrane and formation of blebs. (Perfusion fixation; uranyl acetate, ×7500)





Figures 4 and 5—Heart perfused for 15 minutes with standard medium, followed by 4 minutes of calcium-free perfusion and 30 seconds of reperfusion with calcium-containing medium. Both figures show contracted myofibrils and contraction bands. The mitochondria have either an electron-opaque matrix (Figure 4) or a swollen appearance (Figure 5). (Perfusion fixation; uranyl acetate, ×7500)



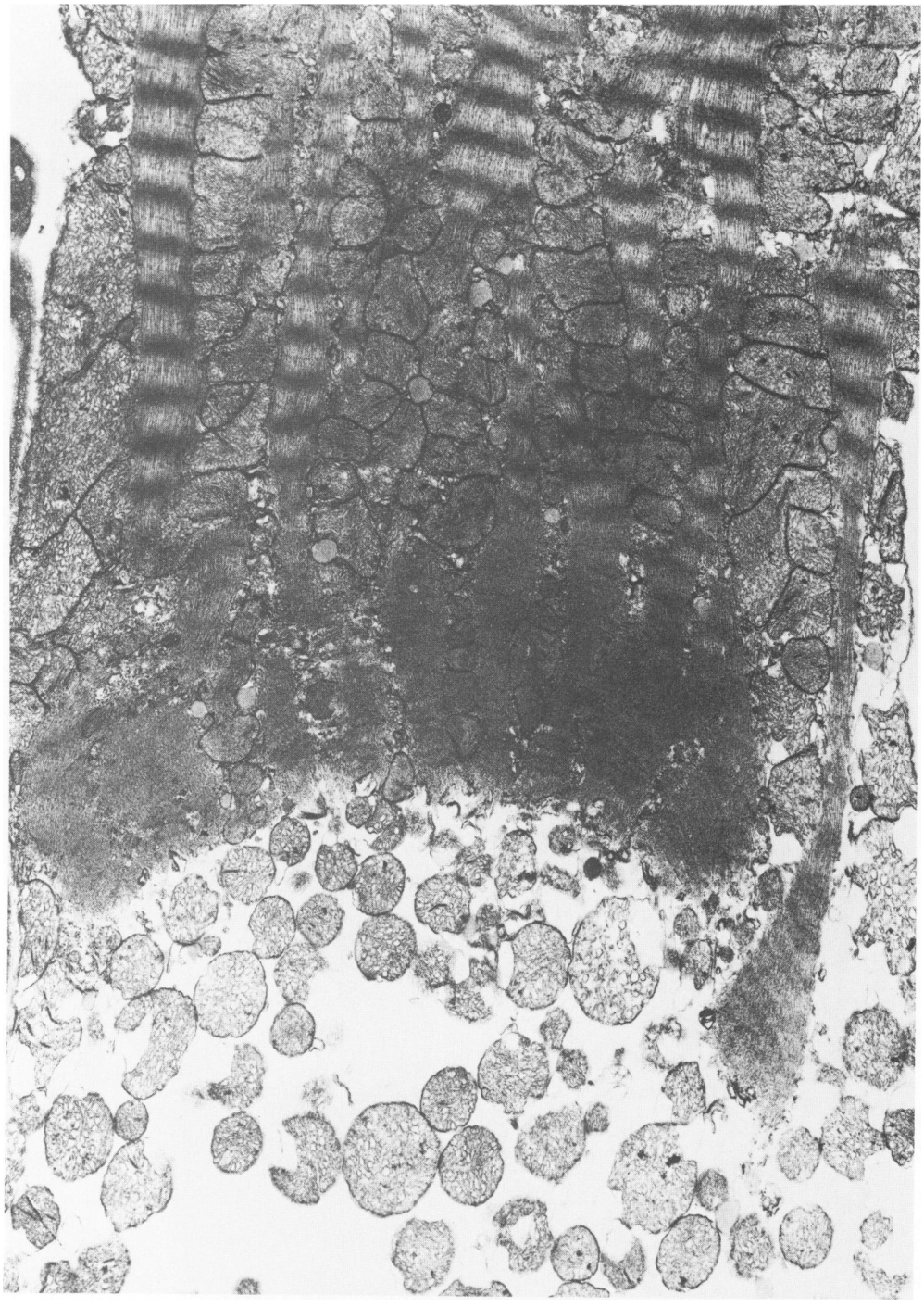


Figure 6—Heart perfused for 15 minutes with standard medium, followed by 4 minutes of calcium-free perfusion and 30 minutes of reperfusion with calcium-containing medium. The calcium-free medium and the reperfusion medium contained 1 mg/l verpamil. This cell shows severe contracture of the myofibrils. The mitochondria are swollen and contain electron-dense material. (Perfusion fixation; uranyl acetate, ×7500)

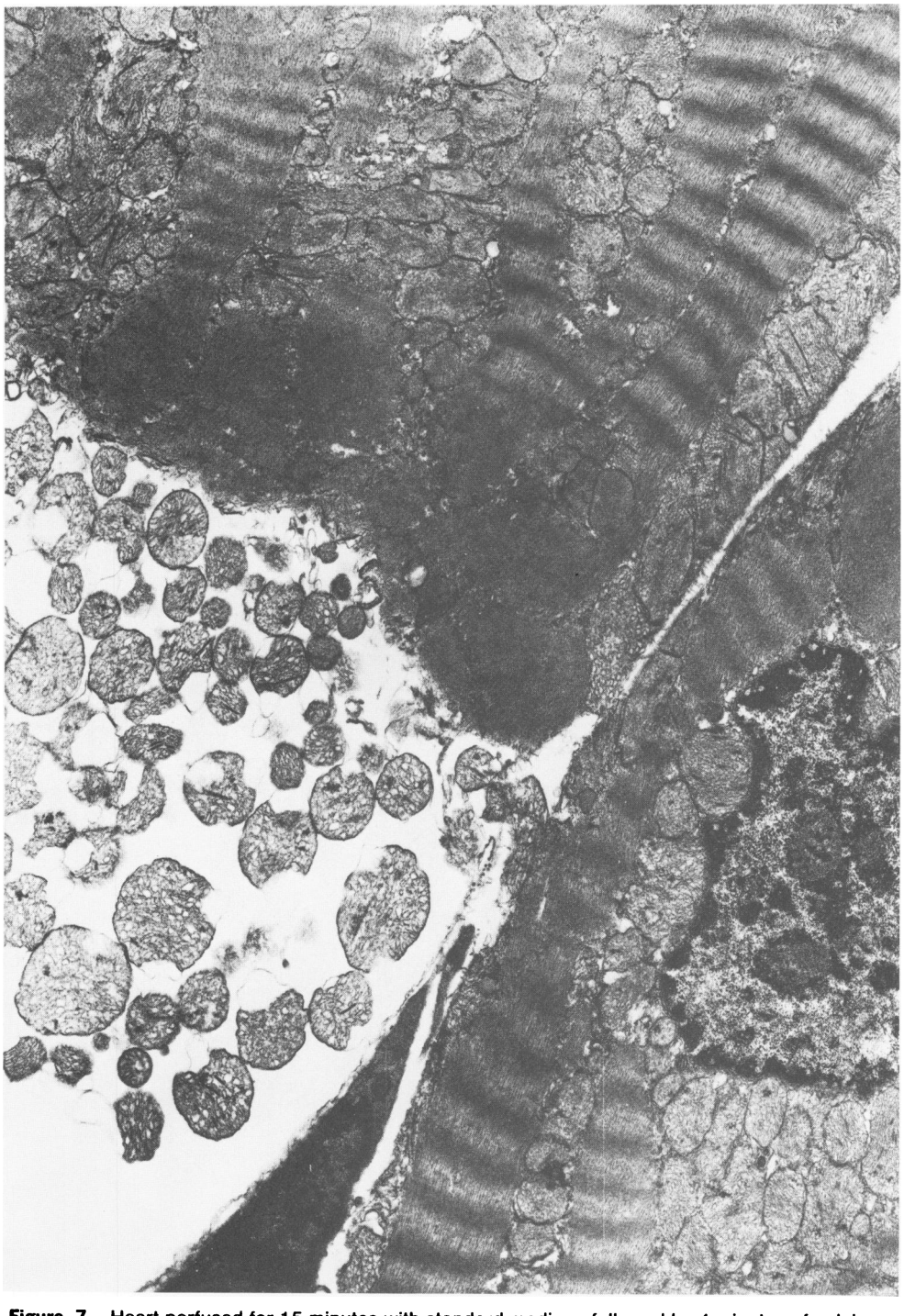


Figure 7—Heart perfused for 15 minutes with standard medium, followed by 4 minutes of calcium-free perfusion and 30 minutes of reperfusion with calcium-containing medium. Myofibrils are heavily contracted; mitochondria are swollen and contain electron-dense deposits. (Perfusion fixation; uranyl acetate, ×7500)

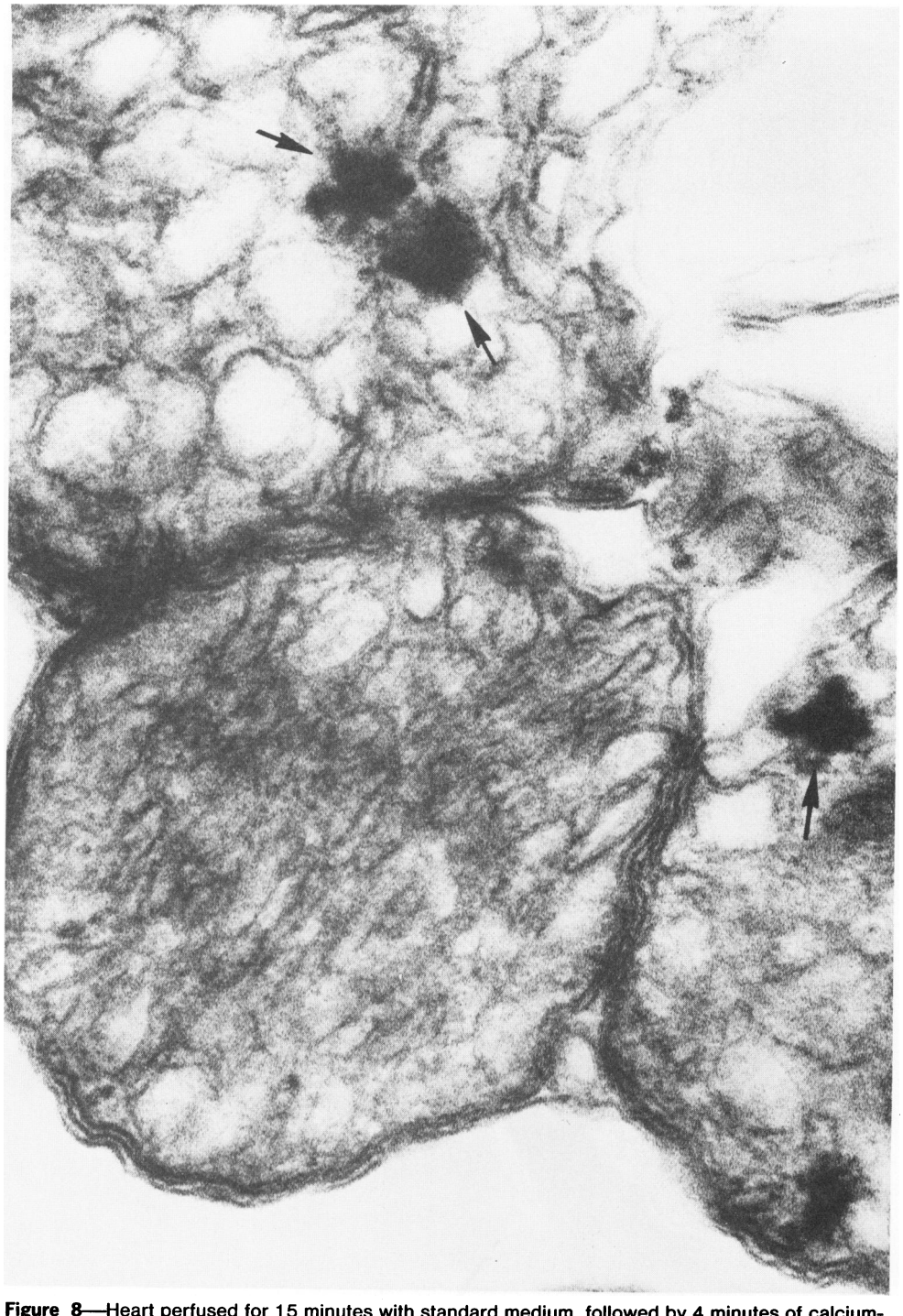


Figure 8—Heart perfused for 15 minutes with standard medium, followed by 4 minutes of calcium-free perfusion and 30 minutes of reperfusion with calcium-containing medium. Mitochondria are swollen and contain electron-dense deposits (*arrows*), which are composed of smaller particles. (Perfusion fixation; uranyl acetate, ×120,000)