American Journal of Pathology, Vol. 131, No. 1, April 1988

Calcium Gain During Postischemic Reperfusion

The Effect of 2,4-Dinitrophenol

JENNIFER S. ELZ, BSc (HONS), and
WINIFRED G. NAYLER, DSc

From the Department of Medicine, University of Melbourne, Austin Hospital, Heidelberg, Victoria, Australia

Reperfusing hearts after an ischemic episode can result in cellular Ca^{2+} overload. This is accompanied by the formation of contraction bands, loss of sarcolemmal integrity, and mitochondrial disruption. The present study investigated the effect of uncoupling oxidative phosphorylation with 2,4-dinitrophenol (DNP) during reperfusion after 30 or 60 minutes of ischemia on this reperfusion-induced Ca^{2+} gain. After 60 minutes' ischemia, reperfusion with 1 mM DNP delayed the accumulation of Ca^{2+} and increased the duration of reperfusion before sarcolemmal disruptions were evident. This suggested that once sarcolemmal integrity is lost, Ca^{2+} will freely enter the cells irrespective of whether the mitochondria are able to accumulate Ca^{2+}. After 30 minutes ischemia, reperfusion for up to 30 minutes with 0.1 or 1 mM DNP attenuated the Ca^{2+} gain and maintained sarcolemmal integrity. Because the authors previously found that maintaining sarcolemmal integrity alone does not totally abolish Ca^{2+} gain, it is suggested that DNP must prevent the entry of Ca^{2+} that occurs via route(s) other than those created by the loss of sarcolemmal integrity. (Am J Pathol 1988, 131: 137–145)

REPERFUSION of an isolated rat heart after 30 or more minutes of ischemia results in a sudden and massive exacerbation of the damage caused by the ischemic episode.[1] The gross morphologic changes that occur under these conditions include contraction band formation, disruption of the orderly arrangement of the myofibrils, swelling of the sarcoplasmic reticulum, loss of sarcolemmal integrity, and vacuolization and disruption of the mitochondria.[2,3] At the same time, intracellular enzymes leak out of the cells, and Ca^{2+} accumulates within the myocytes.[4–8] Similar changes occur upon reoxygenation after a prolonged period of hypoxia.[9]

Precisely why postischemic reperfusion or posthypoxic reoxygenation causes an uncontrolled gain in Ca^{2+} is unknown, nor is there any certainty that the cause is the same in both instances. In the case of posthypoxic Ca^{2+} gain, the fact that the Ca^{2+} gain is minimized by either uncoupling oxidative phosphorylation with 2,4-dinitrophenol (DNP) or inhibiting respiration with cyanide has caused some investigators to argue that the active accumulation of Ca^{2+} by the mitochondria plays a dominant role in precipitating Ca^{2+} overload under these conditions.[10] An alternative argument is that the reoxygenation-induced repletion of adenosine triphosphate (ATP) reserves triggers a rapid and uncontrolled contracture, which in turn generates abnormal physical stresses and disruption of the sarcolemma.[11] This loss of sarcolemmal integrity would then allow Ca^{2+} to freely enter the cells and intracellular enzymes to escape.

The experiments presented here were undertaken to establish whether DNP, which has been shown to prevent contraction band formation, enzyme release, and Ca^{2+} overload during posthypoxic reoxygenation,[10,11] acts in a similar way when added during postischemic reperfusion. Cyanide was not used because in pilot studies we confirmed a previously reported observation that a considerable length of time is required for cyanide to completely block respiration in isolated, perfused hearts.[12] Our experimental protocol involved adding DNP upon reperfusion after 30 or 60

Supported by a grant from the National Health and Medical Research Council of Australia.

Address reprint requests to Dr. W. G. Nayler, Department of Medicine, Austin Hospital, Heidelberg, Victoria, 3084 Australia.

minutes' ischemia and monitoring the Ca^{2+} gain and ultrastructural changes during the course of reperfusion.

Materials and Methods

Model

Adult female Sprague–Dawley rats (180–250 g) were used for these experiments. They were anesthetized with a diethylether/O_2 mixture. The heart was then rapidly excised and placed in ice-cold Krebs-Henseleit buffer until contractions ceased. The heart was then attached to a stainless steel cannula with a tie placed around the aorta and subjected to a nonrecirculating Langendorff perfusion at 37 C and at a constant flow of 10 ml/min.[13,14] The hearts were allowed to beat spontaneously.

Perfusion Media

Krebs-Henseleit buffer (KH) contained NaCl, 119.0 mM; $NaHCO_3$, 25.0 mM, KCl, 4.6 mM, KH_2PO_4, 1.2 mM, $MgSO_4$, 1.2 mM, $CaCl_2$, 1.3 mM; and glucose, 11.0 mM.

DNP-KH was prepared by adding DNP to KH for a final concentration of 0.1 or 1 mM. All perfusion buffers were gassed with 95% O_2/5% CO_2 to provide a pH of 7.4.

Perfusion Sequences

Control Series

Hearts were subjected to an aerobic perfusion of 120 minutes with KH at 37 C. To investigate the effect of DNP on these aerobically perfused hearts, we performed a second series of experiments in which 0.1 or 1 mM DNP was added for the final 30 minutes.

Ischemia Series

After an initial equilibration of 30 minutes' aerobic perfusion with KH at 37 C, hearts were made globally ischemic at 37 C by cessation of all coronary flow for 30 or 60 minutes. The temperature was maintained during ischemia by surrounding water-heated jackets.

Reperfusion Series

After 30 or 60 minutes' global ischemia, the hearts were reperfused for 5, 10, 15, or 30 minutes with KH or KH containing 0.1 or 1 mM DNP at 37 C. In one series, hearts were reperfused with 0.1 mM DNP for 60 or 120 minutes after 30 minutes of ischemia.

Four to six hearts were perfused according to each sequence, and they were then analyzed for Ca^{2+} content and reflow area, or they were perfusion-fixed for microscopy as described below.

Ca^{2+} Analysis

After perfusion, the hearts were flushed with 10 ml of an ice-cold 0.35 M sucrose, 5 mM histidine solution, pH 7.4, which had been pretreated with Dowex cation exchange resin (50W) for minimization of the contribution of extracellular Ca^{2+} to the measured cell Ca^{2+}.[15] The Ca^{2+} content was measured as previously described.[14] Briefly, the atria were discarded and the ventricles dried to constant weight at 100 C. The dried ventricles were then digested in concentrated nitric acid and diluted in a blank solution containing 27 mM KCl and 27 μM $LaCl_3$. The Ca^{2+} content was determined with a Varian atomic absorption spectrophotometer at 422.7 nm and compared with a standard curve produced by serial dilution of a 1 M Ca^{2+} solution (BDH Chemicals, Port Fairy, Victoria, Australia) in the above blank solution. This method gives an estimation of the total cellular calcium content. It does not detect movements in intracellular Ca^{2+}, nor is it sensitive enough to measure micromolar changes in Ca^{2+} content.

Reflow Area

At the conclusion of the perfusion sequence 2.4 ml of the histologic stain carbol fuchsin was passed through the coronary vasculature for determining the percentage of the ventricles being perfused. The hearts were removed from the perfusion apparatus, suspended in plastic containers, and frozen at −80 C. When frozen, free-hand transverse sections were cut with a razor blade, and the areas of reflow and no-reflow were traced onto glass slides. The areas of reflow were stained dark red, whereas the areas of no-reflow remained pale. The percentage of the ventricles receiving flow—ie, the "reflow area"—was quantified by point counting using a 1-mm square lattice.[16] It was expressed as a percentage of the cross-sectional area of the ventricles.

Pilot experiments demonstrated that this method gives results similar to those of the method of Humphrey et al[17] using fluorescein.

Microscopy

For each required perfusion sequence at least four hearts were perfusion-fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer for 5 minutes. Transverse

slices of the left ventricle were cut by hand and small blocks of tissue immersed in the fixative for a further 2 hours. After thorough rinsing in buffer, six to eight tissue blocks were postfixed with osmium tetroxide, dehydrated through graded concentrations of acetone, and infiltrated with and embedded in araldite/Epon.

Sections were cut and stained with methylene blue for light microscopy. These sections were used for identification of representative areas, and the blocks were trimmed to encompass these areas. Thin sections were cut on an LKB III ultramicrotome, mounted on copper grids, and stained with uranyl acetate and lead citrate. Six to eight sections were examined and photographed with a JEOL 1200 EX transmission electron microscope.

Statistical Analysis

Results are expressed as the mean ± standard error of the mean (SEM) of six separate experiments unless stated otherwise. Tests of significance were performed using a one-way analysis of variance followed by Tukey's multiple comparison test, and $P = 0.05$ was taken as the limiting level of significance.

Results

Aerobic Perfusion

The introduction of 0.1 or 1 mM DNP to aerobically perfused hearts resulted in the rapid cessation of contractile activity. Removal of DNP from the perfusion buffer did not result in a return of active tension generation, which implied that DNP cannot be washed out of the heart.

After 90 minutes' KH perfusion the hearts contained 5.22 ± 0.44 μmol Ca^{2+}/g dry wt. This level was not altered by continuing aerobic KH perfusion for a further 30 minutes (Figure 1). Nor was it altered if 0.1 or 1 mM DNP was introduced for the final 30 minutes (Figure 1).

After 30 minutes' aerobic KH perfusion, the myofibrils were relaxed and displayed prominent I bands (Figure 2a). Mitochondria were distributed between the myofibrils and each cell was surrounded by an intact sarcolemmal complex (Figure 2a). If 1 mM DNP was introduced, the myofibrils contracted such that the I bands were no longer apparent (Figure 2b).

Ischemia

Neither 30 nor 60 minutes of ischemia had any effect on the Ca^{2+} content of the hearts. The ultrastructural appearance of the hearts after 30 or 60 minutes of ischemia was similar. The myofibrils were contracted such that the I bands were no longer apparent, the glycogen stores were depleted, and the nuclear chromatin was clumped at the periphery of the nucleus (Figure 3). Sarcolemmal discontinuities were not apparent in cell profiles of sections examined with high-power electron microscopy after either 30 or 60 minutes of ischemia. This differs from the results reported by other investigators and may be indicative of the differences that may occur when different species and models of ischemia are used.[18,19]

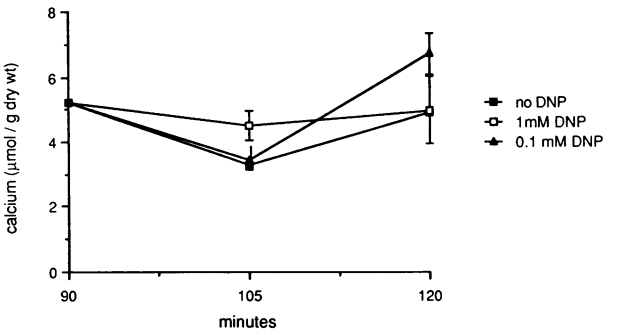

Figure 1—The effect of 0.1 or 1 mM DNP on the Ca^{2+} content of hearts aerobically perfused with KH. All hearts were perfused with KH for 90 minutes. The perfusion then was continued for a further 30 minutes with KH or with KH containing 0.1 or 1 mM DNP. Each point represents the mean ± SEM of four to six separate experiments.

Reperfusion

After 30 or 60 minutes ischemia, not all of the ventricles were reperfused—the "no-reflow" phenomenon.[17] DNP had no effect on the percentage of the ventricles receiving flow after 30 or 60 minutes of ischemia. Ten minutes' reperfusion after 30 minutes ischemia resulted in 78% ± 3% and 70% ± 3% of the ventricles being reperfused in the absence or presence of DNP, respectively. After 60 minutes ischemia, 64% ± 3% of the ventricles were reperfused after 10 minutes and 61% ± 5% after 30 minutes' reperfusion with KH. These values were not altered in the presence of DNP, being 62% ± 2% after 10 minutes and 65% ± 4% after 30 minutes' reperfusion.

Reperfusion of hearts with KH after 60 minutes of ischemia resulted in an accumulation of Ca^{2+}, peaking after 15 minutes reperfusion (Figure 4a). When DNP was added to the reperfusion medium to a final concentration of 0.1 mM, there was no effect on the Ca^{2+} accumulated after 60 minutes of ischemia (Figure 4a). However, if the hearts were reperfused with 1 mM DNP-KH, Ca^{2+} uptake was delayed, such that

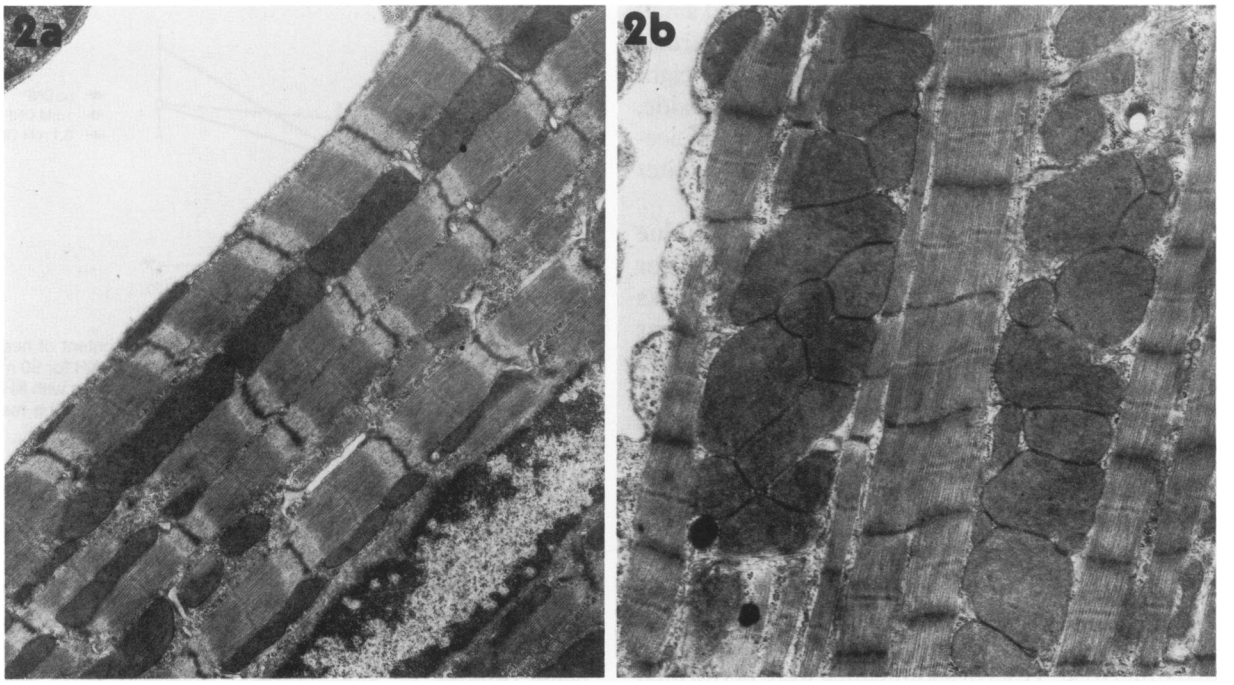

Figure 2—Electron micrographs from hearts aerobically perfused in the absence (**a**) or presence (**b**) of 1 mM DNP. **a**—Note the regular arrangement of the myofibrils, the mitochondria with condensed cristae, and the intact sarcolemma. (×9500) **b**—In the presence of DNP, the myofibrils were contracted such that the I bands were no longer apparent. (×12,000)

there was little Ca^{2+} gained during the initial 10 minutes of reperfusion. Thereafter, the Ca^{2+} levels increased; and after 30 minutes' reperfusion with DNP, there was no difference from the Ca^{2+} level obtained in the absence of DNP (Figure 4a).

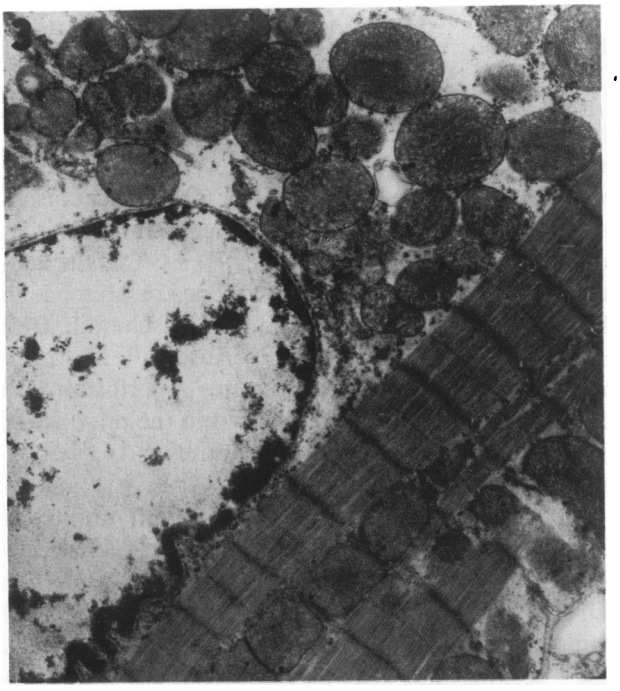

Figure 3—Electron micrograph from a heart after 60 minutes ischemia. Note the clumped nuclear chromatin, the loss of glycogen, and the contracted myofibrils. (×9500)

Reperfusion with KH after 30 minutes of ischemia also resulted in an accumulation of Ca^{2+} by the hearts (Figure 4b). These hearts appeared to accumulate more Ca^{2+} than those reperfused after 60 minutes of ischemia. One possible explanation for this is that more cells are exposed to the reperfusion medium after 30 than after 60 minutes of ischemia, as indicated by the percentage of the ventricles receiving flow (discussed earlier). Hence, if all cells accumulate a maximum amount of Ca^{2+}, the amount measured after 30 minutes of ischemia will be greater by virtue of a greater number of cells being involved. When hearts were reperfused with 0.1 or 1 mM DNP-KH after 30 minutes of ischemia, the Ca^{2+} gain was attenuated throughout the entire 30 minutes' reperfusion (Figure 4b). Extending the period of reperfusion with 0.1 mM DNP after 30 minutes of ischemia resulted in a gain in Ca^{2+} to 11.33 ± 0.60 μmol/g dry wt ($n = 4$) after 60 minutes and 19.27 ± 0.94 μmol/g dry wt ($n = 4$) after 120 minutes' reperfusion.

After 60 minutes' ischemia, reperfusion with KH for 5 minutes resulted in ultrastructural damage. The cells showed a discontinuous sarcolemma, some contraction bands, and swollen mitochondria. As the length of reperfusion was increased, the number of cells with contraction bands increased, and the dam-

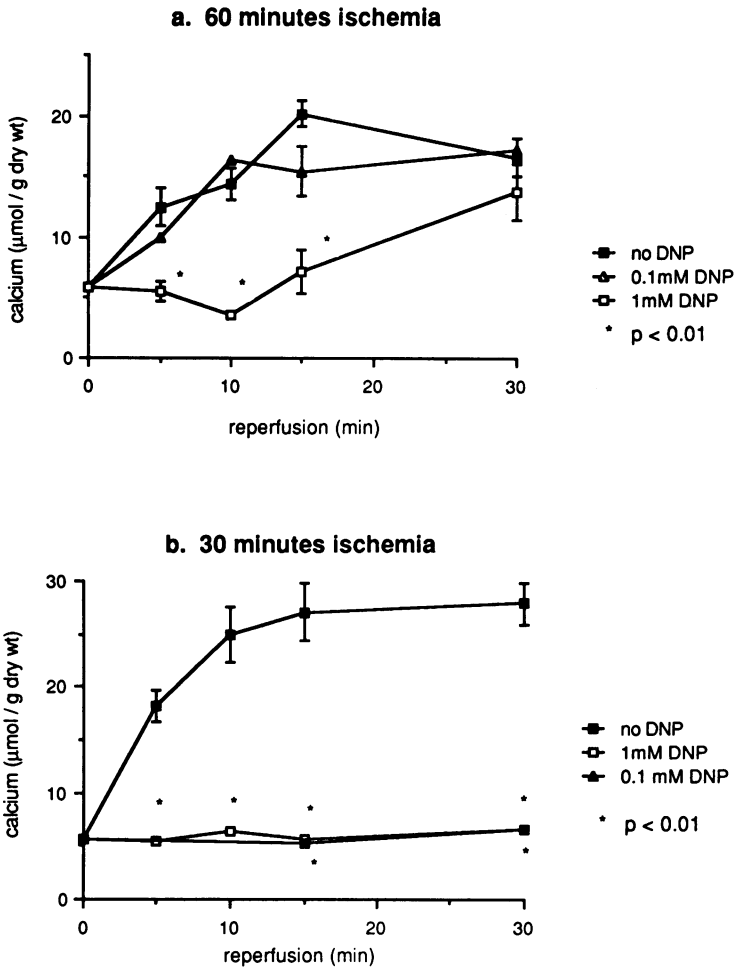

Figure 4—The effect of DNP, added during reperfusion, after 60 (**a**) or 30 (**b**) minutes of ischemia on the reperfusion-induced Ca^{2+} gain. Each point represents the mean ± SEM of six separate experiments. Statistics refer to the Ca^{2+} content in the presence of DNP, compared with that in its absence.

age to the sarcolemma became more evident (Figure 5a). When the hearts were reperfused with 1 mM DNP-KH, contraction bands were not observed at any time during reperfusion. Sarcolemmal integrity appeared to be maintained for the initial 10 minutes of reperfusion with 1 mM DNP. Thereafter, sarcolemmal discontinuities became apparent (Figure 5b).

Reperfusion with KH after 30 minutes ischemia resulted in a ultrastructural appearance similar to that found with reperfusion after 60 minutes of ischemia. Contraction bands were prominent, the mitochondria were swollen, and the integrity of the sarcolemma was lost (Figure 6a). When hearts were reperfused with 1 mM DNP-KH after 30 minutes ischemia, the sarcolemmal complex remained intact surrounding contracted myofibrils throughout 30 minutes' reperfusion (Figure 6b).

Discussion

This study investigated whether the uncoupling of oxidative phosphorylation with DNP affects the reperfusion-induced Ca^{2+} gain following 30 or 60 minutes of ischemia in the isolated perfused rat heart. DNP uncouples oxidative phosphorylation, causes a release of the mitochondrial Ca^{2+} stores, and prevents any further mitochondrial accumulation of Ca^{2+}.[20,21] The mitochondrial Ca^{2+} release and the abrupt decline in ATP levels are associated with the development of contracture.[22,23] The mitochondrial release of Ca^{2+} would be expected to be associated with a rise in cytoplasmic Ca^{2+}. In addition, because ATP is neither available to facilitate uptake of Ca^{2+} by the sarcoplasmic reticulum nor to facilitate removal of Ca^{2+} from the cytoplasm across the sarcolemma via ATP-dependent mechanisms, this would also promote an increase in cytoplasmic Ca^{2+}. Our technique of Ca^{2+} analysis does not allow us to detect such changes.

The attenuation of the Ca^{2+} gain in the presence of DNP does not appear to be the result of a reduced area of the ventricles receiving flow. However, it is possible that a reduction in the area of reflow during reperfusion in the absence of DNP is responsible for the decreased Ca^{2+} gain following 60 minutes of ischemia,

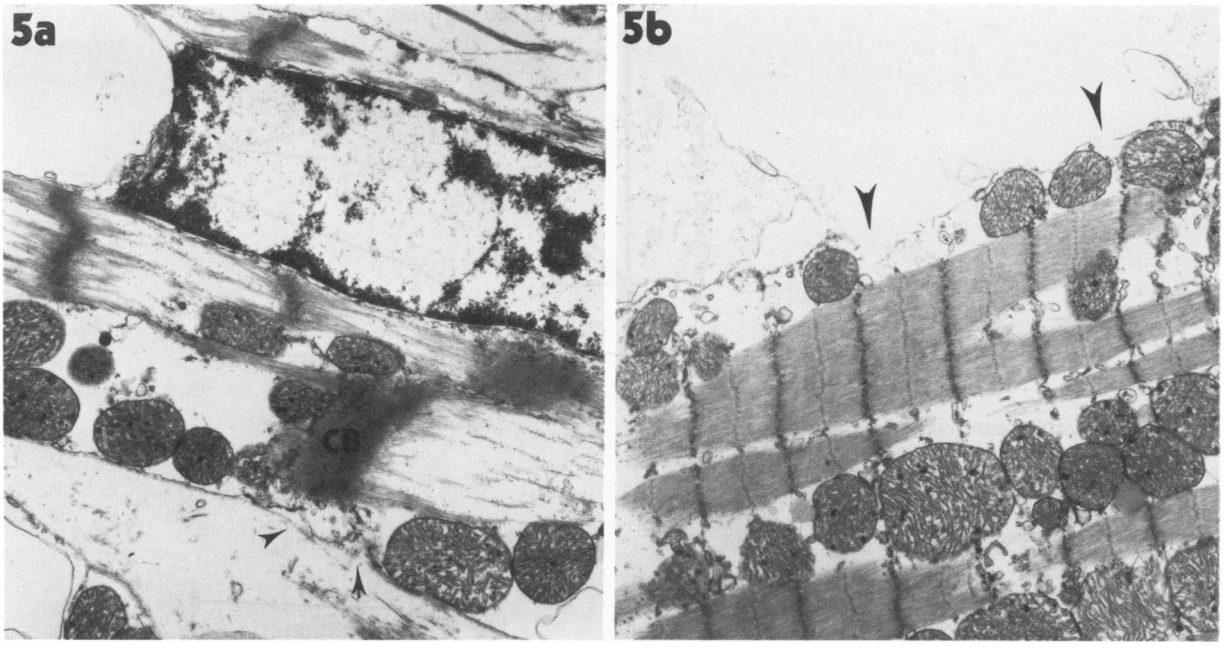

Figure 5—Electron micrographs from hearts after 30 minutes' reperfusion in the absence (**a**) or presence (**b**) of 1 mM DNP after 60 minutes' ischemia. **a**—Note the contraction bands (*CB*) and sarcolemmal disruptions (*arrowheads*). (×9500) **b**—Contraction bands were not found in the presence of DNP, but the sarcolemmal integrity was still lost (*arrowheads*). (×9500)

compared with that found after 30 minutes of ischemia (Figure 4).

Following 30 minutes of ischemia, the addition of 0.1 or 1 mM DNP to the reperfusion buffer abolished the gain in Ca^{2+} found in the absence of DNP during 30 minutes' reperfusion. It is possible that cytoplasmic Ca^{2+} may have risen during this time, but this would not be detected by the method of Ca^{2+} mea-

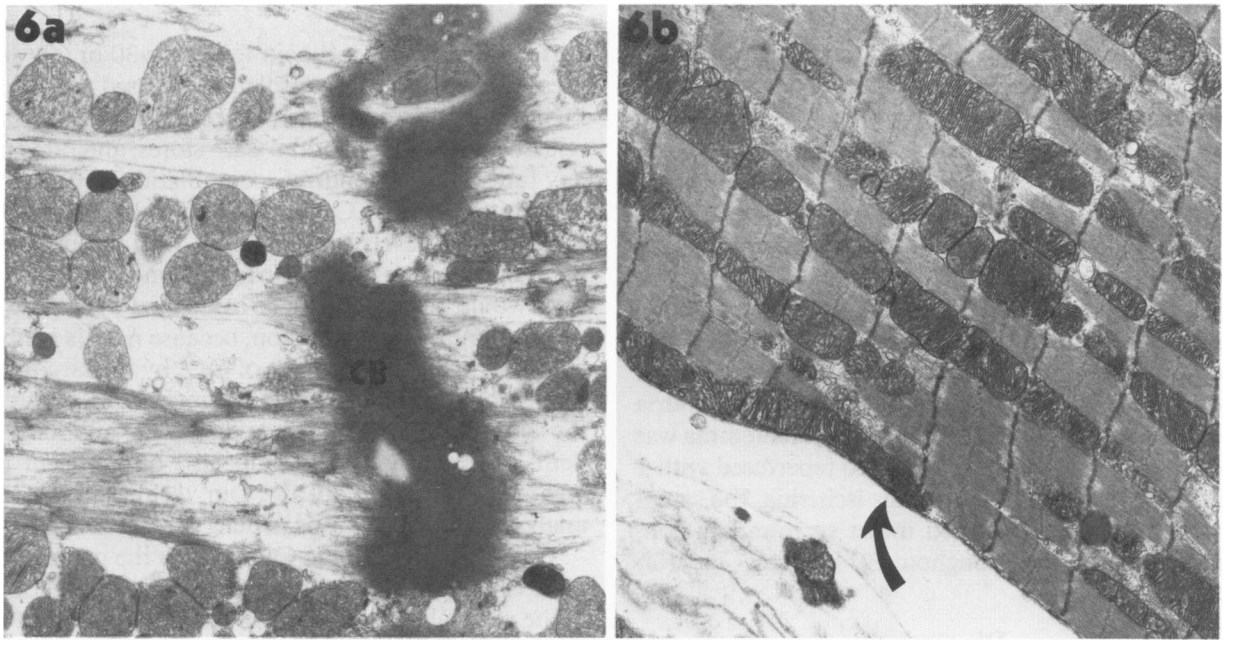

Figure 6—Electron micrographs from hearts after 30 minutes' reperfusion in the absence (**a**) or presence (**b**) of 1 mM DNP after 30 minutes' ischemia. **a**—Note the contraction bands (*CB*). (×9500) **b**—In the presence of DNP, contraction bands were not observed, and the sarcolemma remained intact (*arrow*). (×8000)

surement used here. One millimolar DNP prevented the loss of sarcolemmal integrity that was seen in its absence during reperfusion following 30 minutes of ischemia. A simple explanation for the lack of a massive Ca^{2+} gain during reperfusion would therefore be the maintenance of an intact sarcolemma. However, in a recent series of experiments using 2,3-butanedione monoxime to inhibit contractile activity during reperfusion following 30 minutes of ischemia, the sarcolemma was maintained in an intact state, but the Ca^{2+} gain was only reduced and not abolished (unpublished observations). These results suggested that the reperfusion-induced Ca^{2+} gain involved entry of Ca^{2+} via route(s) other than those associated with a disrupted sarcolemma. In the present series of experiments DNP has inhibited the entry of Ca^{2+} via routes associated and not associated with sarcolemmal "holes." Those routes not associated with a ruptured sarcolemma could include entry of Ca^{2+} via passive diffusion, through the slow channels or in exchange for Na^+. The entry of Ca^{2+} via passive diffusion seems unlikely, because experiments on isolated sarcolemmal membranes do not show an increase in passive Ca^{2+} permeability.[24] The entry of Ca^{2+} via the slow channels is also unlikely, because the addition of slow channel blockers during reperfusion has little or no effect on the gain in Ca^{2+}.[25] Entry of Ca^{2+} in exchange for Na^+ has previously been suggested as a route of Ca^{2+} entry following ischemia or hypoxia, and it is possible that the accumulation of Ca^{2+} by the mitochondria may maintain an inward gradient for Ca^{2+}, which makes this a continuing process.[26,27] DNP may directly inhibit the Na^+/Ca^{2+} exchange mechanism, or, alternatively, by preventing mitochondrial Ca^{2+} accumulation, may decrease the inward gradient to Ca^{2+}.[10]

After 60 minutes of ischemia 0.1 mM DNP had no effect on the reperfusion-induced Ca^{2+} gain, and 1 mM DNP delayed, but did not abolish, the gain. Ca^{2+} entry in the presence of 1 mM DNP appeared to coincide with the appearance of sarcolemmal discontinuities, which suggested that Ca^{2+} may be freely entering the cells through these "holes." The attainment of Ca^{2+} levels in the presence of 1 mM DNP that are equivalent to those in its absence suggests that mitochondrial Ca^{2+} accumulation is not essential for Ca^{2+} overloading of the cells when sarcolemmal integrity has been lost. Thus, DNP may attenuate the reoxygenation-induced Ca^{2+} gain following hypoxia by preventing mitochondrial accumulation of Ca^{2+}, only if the sarcolemma is intact.[10]

The question that remains is, why is it that the sarcolemmal integrity is lost during reperfusion following 60, but not 30, minutes of ischemia? One possible explanation is based on the work of Jennings et al.[28] These workers proposed that the accumulation of breakdown products from ischemic metabolism may increase cellular osmolarity. This, in turn, may contribute to cell swelling, which will place added stress on the sarcolemma and may result in its disruption. In our model of global ischemia with no collateral flow, it is unlikely that sufficient cell swelling will occur during the ischemic episode to rupture the cell membrane. This is supported by the ultrastructural results in the present experiments, in which sarcolemmal discontinuities were not evident at the end of the ischemic episode. However, it is likely that during reperfusion cell swelling may place extra stress on the sarcolemma. Then, with the added stress caused by the resumption of contractile activity, complete rupture of the membrane could occur, allowing a rapid and uncontrolled entry of Ca^{2+}. In the presence of 1 mM DNP, ATP is not available, and hence contractile activity does not resume.

After 30 minutes of ischemia the integrity of the sarcolemma is maintained throughout 30 minutes' reperfusion in the presence of 1 mM DNP, which suggests that the sarcolemma can withstand the stress imposed on it by cell swelling due to the increase in intracellular osmolarity as a result of the accumulation of breakdown products during ischemia. However, disruption of the sarcolemma was evident after 15 minutes' reperfusion with DNP-KH after 60 minutes' ischemia. It is possible to envisage that the increased duration of the ischemic episode will result in a greater increase of intracellular osmolarity, and this in turn will cause greater cell swelling, which may ultimately result in the development of sarcolemmal "holes." Once present, these holes will almost certainly represent lethal cellular injury with the accumulation of an uncontrolled amount of Ca^{2+}. However, if the development of sarcolemmal discontinuities is a result of swelling induced by the accumulation of metabolites during the ischemic episode, we would expect the membrane damage to occur early during reperfusion and not after 15 minutes, when all osmotically active metabolites should have been washed from the cells.

An alternative and possibly more likely explanation is that reperfusion in the presence of DNP may merely be extending the length of time hearts are effectively without usable O_2—ie, the hearts are exposed to 30 or 60 minutes of ischemia, followed by 30 minutes of a biochemically induced hypoxia. Reperfusion with DNP after ischemia will result in a return of flow with the detrimental effects associated with cell swelling

and a return of O_2, which although not able to be used to produce energy via oxidative phosphorylation, may provide a source of free radicals.[29] These factors, together with alterations to the membrane phospholipids, which become more severe with increasing durations of ischemia,[30,31] may contribute to the development of membrane damage. Our results support this hypothesis. As we would expect, a longer period of DNP reperfusion was required to produce damage after 30 minutes of ischemia than after 60 minutes. Whereas 15 minutes' reperfusion following 60 minutes of ischemia was sufficient to result in a gain in Ca^{2+}, more than 30 minutes' reperfusion with DNP was required after 30 minutes of ischemia.

In summary, these results have illustrated that sarcolemmal integrity must be maintained if the reperfusion-induced gain in Ca^{2+} is to be attenuated. The loss of sarcolemmal integrity during reperfusion following an ischemic episode may be the result of multiple causes, one of which is probably the stress placed on a susceptible membrane by the return of contractile activity. Maintaining sarcolemmal integrity, however, is not sufficient to totally abolish the reperfusion-induced Ca^{2+} gain (unpublished observations). In the presence of DNP, reperfusion for up to 30 minutes following 30 minutes of ischemia resulted in a maintenance of the sarcolemma in an intact state and an abolition of the Ca^{2+} gain, which indicates that DNP was able to inhibit the Ca^{2+} entry via route(s) other than those associated with entry through sarcolemmal "holes." This inhibition may relate to DNP preventing mitochondrial Ca^{2+} accumulation and hence removing the possibility of a continuous inward gradient for Ca^{2+} being established.

References

1. Hearse DJ: Reperfusion of the ischemic myocardium. J Mol Cell Cardiol 1977, 9:605–616
2. Herdson PB, Kaltenbach JP, Jennings RB: Fine structural and biochemical changes in dog myocardium during autolysis. Am J Pathol 1969, 57:539–557
3. Kloner RA, Ganote CE, Whalen DA Jr, Jennings RB: Effect of a transient period of ischemia on myocardial cells: II. Fine structure during the first few minutes of reflow. Am J Pathol 1974, 74:399–422
4. Humphrey SM, Thomson RW, Gavin JB: The influence of the no-reflow phenomenon on reperfusion and reoxygenation damage and enzyme release from anoxic and ischaemic isolated rat hearts. J Mol Cell Cardiol 1984, 16:915–930
5. Kent SP: Intracellular plasma protein: A manifestation of cell injury in myocardial ischemia. Nature 1966, 210: 1279–1281
6. Shen AC, Jennings RB: Myocardial calcium and magnesium in acute ischemic injury. Am J Pathol 1972, 67: 417–440
7. Shen AC, Jennings RB: Kinetics of calcium accumulation in acute myocardial ischemic injury. Am J Pathol 1972, 67:441–452
8. Shine KI, Douglas AM, Ricchiuti NV: Calcium, strontium and barium movements during ischemia and reperfusion in rabbit ventricle: Implications for myocardial preservation. Circ Res 1978, 43:712–720
9. Ganote CE, Seabra-Gomes R, Nayler WG, Jennings RB: Irreversible myocardial injury in anoxic perfused rat hearts. Am J Pathol 1975, 80:419–450
10. Nakanishi T, Nishioka K, Jarmakani JM: Mechanism of tissue Ca^{2+} gain during reoxygenation after hypoxia in rabbit myocardium. Am J Physiol 1982, 242:H437–H449
11. Ganote CE, McGarr J, Liu SY, Kaltenbach JP: Oxygen-induced enzyme release: Assessment of mitochondrial function in anoxic myocardial injury and effects of the mitochondrial uncoupling agent 2,4-dinitrophenol (DNP). J Mol Cell Cardiol 1980, 12:387–408
12. Ganote CE, Worstell J, Kaltenbach JP: Oxygen-induced enzyme release after irreversible myocardial injury: Effects of cyanide in perfused rat hearts. Am J Pathol 1976, 84:327–350
13. Langendorff O: Untersuchungen am uberlebenden Saugetierherzen. Pflugers Arch Ges Physiol 1895, 61: 291–339
14. Nayler WG, Perry SE, Elz JS, Daly MJ: Calcium, sodium, and the calcium paradox. Circ Res 1984, 55:227–237
15. Alto LE, Dhalla NS: Myocardial cation contents during induction of calcium paradox. Am J Physiol 1979, 237: H713–H719
16. Weibel ER, Kistler GS, Scherie VF: Practical stereological methods for morphometric cytology. J Cell Biol 1966, 30:23–38
17. Humphrey SM, Gavin JB, Herdson PB: The relationship of ischemic contracture to vascular reperfusion in the isolated rat heart. J Mol Cell Cardiol 1980, 12:1397–1406
18. Jennings RB, Ganote CE: Structural changes in myocardium during acute ischemia. Circ Res 1974, 34/35 (Suppl III):156–168
19. Jennings RB, Ganote CE, Reimer KA: Ischemic tissue injury. Am J Pathol 1975, 81:179–198
20. Wilson DF, Ting HP, Koppelman MS: Mechanism of action of uncouplers of oxidative phosphorylation. Biochemistry 1971, 10:2897–2902
21. Deth R, Casteels R: A study of releasable Ca fractions in smooth muscle cells of the rabbit aorta. J Gen Physiol 1977, 69:401–416
22. Ganote CE, Grinwald PM, Nayler WG: 2,4-Dinitrophenol (DNP) induced injury in calcium-free hearts. J Mol Cell Cardiol 1984, 16:547–557
23. Vander Heide RS, Angelo JP, Altschuld RA, Ganote CE: Energy dependence of contraction band formation in perfused hearts and isolated adult myocytes. Am J Pathol 1986, 125:55–68
24. Daly MJ, Elz JS, Nayler WG: Sarcolemmal enzymes and Na^+Ca^{2+} exchange in hypoxic, ischemic and reperfused rat hearts. Am J Physiol 1984, 247:H237–H243

25. Bourdillon PDV, Poole-Wilson PA: The effects of verapamil, quiescence and cardioplegia on calcium exchange and mechanical function in ischemic rabbit myocardium. Circ Res 1982, 50:360–368
26. Grinwald PM, Brosnahan C: Sodium imbalance as a cause of calcium overload in post-hypoxic reoxygenation injury. J Mol Cell Cardiol 1987, 19:487–495
27. Renlund DG, Gerstenblith G, Lakatta EG, Jacobus WE, Kallman CH, Weisfeldt ML: Perfusate sodium during ischemia modifies postischemic functional and metabolic recovery in the rabbit heart. J Mol Cell Cardiol 1984, 16:795–801
28. Jennings RG, Reimer KA, Steenbergen C: Myocardial ischemia revisited: The osmolar load, membrane damage, and reperfusion. J Mol Cell Cardiol 1986, 18:769–780
29. Hammond B, Hess ML: The oxygen free radical system: Potential mediator of myocardial injury. J Am Coll Cardiol 1985, 6:215–220
30. Chien KR, Han A, Sen A, Buja LM, Willerson JT: Accumulation of unesterified arachidonic acid in ischemic canine myocardium: Relationship to a phosphatidylcholine deacylation-reacylation cycle and the depletion of membrane phospholipids. Circ Res 1984, 54:313–322
31. Das DK, Engelman M, Rousou JA, Breyer RH, Otani H, Lemeshow S: Role of membrane phopholipids in myocardial injury induced by ischemia and reperfusion. Am J Physiol 1986, 251:H71–H79