The Pathogenesis of Irreversible Cell Injury in Ischemia

JOHN L. FARBER, MD, KENNETH R. CHIEN, MD, and STEWART MITTNACHT, JR., AB

From the Department of Pathology and the Fels Research Institute, Temple University School of Medicine, Philadelphia, Pennsylvania

Cells made ischemic rapidly manifest many distinct structural and functional alterations as a consequence of the depletion of their energy stores. In attempting to determine which of these are causally related to the eventual cell death, the authors have emphasized the relationship to the reversibility of the ischemic injury. Two phenomena have consistently characterized irreversibly in contrast to reversibly injured ischemic cells: the inability to restore mitochondrial function and evidence of plasma membrane damage. Studies in the authors' laboratory are reviewed that have focused on the pathogenesis, biochemical nature, and the relationship to irreversible cell injury of both of these alterations. A number of mitochondrial abnormalities are related to changes in long-chain acyl-CoA metabolism with inhibition of adenine nucleotide translocation and potentiation of a Ca^{2+}-dependent increase in the permeability of the inner mitochondrial membrane. These changes are reversible upon reoxygenation only when the large increase in intracellular Ca^{2+} content that accompanies the phospholipid depletion from other cellular membranes is prevented. This disorder in phospholipid metabolism is felt to be the critical lesion that produces irreversible cell injury in ischemia. It affects the endoplasmic and sarcoplasmic reticular membranes of liver and myocardial cells, respectively, and probably the plasma membranes of both. It is prevented by pretreatment with chlorpromazine. An activation of endogenous phospholipases by an elevated, cytosolic free Ca^{2+} ion concentration is suggested as the mechanism underlying this phospholipid disturbance. The central role of intracellular Ca^{2+} in the initiation and functional consequences of ischemic cell injury are emphasized. (Am J Pathol 1981, 102:271-281)

THE IMPORTANCE of ischemia in human disease makes understanding of the mechanisms whereby it produces irreversible cell injury of considerable interest. Such an understanding, however, has been elusive, and the reason is not obscure. Well-differentiated tissues such as heart, liver, kidney, and brain require large amounts of oxygen to support their various specialized functions. In general, these functions can only be maintained by energy derived from aerobic metabolism and cease when oxygen is in short supply. Ischemia will, therefore, set rapidly into motion a complex series of events that involve virtually every organelle and subcellular system in the affected cells. As cells become anoxic, oxidative phosphorylation ceases. ATP stores are depleted, and virtually all energy-dependent functions cease. There are inhibition of RNA and protein synthesis and changes in ion transport with loss of intracellular K^+ and increased intracellular Na^+ ions. Glycolysis is initially accelerated, and the cells produce large quantities of lactic acid. When glycogen stores are depleted, glycolysis slows. Ischemic heart muscle rapidly ceases to contract.

This variety of cellular alterations would seem to account readily for the fact that ischemia is such a lethal hazard. However, evidence from other studies indicates that many of these alterations by themselves do not result in cell death. In analyzing ischemic cell injury, the task is to identify from among many changes those that are causally related to cell death. Any particular metabolic alteration may be unrelated to the cell death, be an effect of the fact that the cell is dead or dying, or be causally related to the cell death. Analysis of ischemic cell injury requires a mechanism whereby any metabolic alteration can be assigned to one of these categories.

Reversible and Irreversible Ischemic Cell Injury

A major strategy to assess causal significance in ischemia makes use of the concept of reversible and

Presented at the Sixty-fourth Annual Meeting of the Federation of American Societies for Experimental Biology, Anaheim, California, April 1980.

Supported by Grants Am 19154 and CA 12073 from the National Institutes of Health.

Address reprint requests to John L. Farber, MD, Department of Pathology, Temple University Health Sciences Center, 3400 N. Broad St., Philadelphia, PA 19140.

0002-9440/81/0218-0271$01.05

irreversible cell injury. It became apparent quite early that the effects of ischemic injury on myocardial mechanical function, membrane potential, metabolism, and ultrastructure are all reversible if the duration of ischemia is short.[1-4] However, if the ischemia persists for longer periods of time, the affected cells become irreversibly injured. That is, the cells continue to degenerate and become necrotic despite reperfusion with arterial blood. Therefore, all of the metabolic alterations associated with reversible ischemic cell injury are either quantitatively or qualitatively unrelated to the development of irreversible injury. With longer periods of ischemia, there develops some biochemical alteration that distinguishes irreversibly from reversibly injured cells.

Two phenomena have consistently characterized irreversibly, as opposed to reversibly, injured cells in several models of ischemia. First, *in vivo* studies of kidney, liver, heart, and brain, as well as the analysis of several *in vitro* models, have documented that an inability to reverse mitochondrial dysfunction upon reperfusion or reoxygenation correlates with a similar inability to reverse the cell injury in general.[5-11] Such studies have been interpreted as indicating that ischemic cell death is a consequence of irreversible mitochondrial injury.[5,11] Mitochondria develop a series of abnormalities as a consequence of ischemia. Restoration of arterial flow is not followed by restoration of cellular function, because mitochondrial function no longer is possible. One difficulty with this hypothesis is that reperfusion may expose the injured cells to an environment that does not allow recovery of otherwise reversible mitochondrial function. In particular, it has been shown that during reperfusion of irreversibly injured cells, a large influx of Ca^{2+} ions occurs.[12,13] Excess Ca^{2+} ions are known to produce loss of mitochondrial function.[14] It is conceivable that an inability to reverse mitochondrial dysfunction may be the consequence of the flooding of the cell with Ca^{2+}, rather than the consequence *per se* of the specific pattern of mitochondrial dysfunction prior to reflow. A critical test of the role of mitochondrial dysfunction in the pathogenesis of irreversible ischemic injury would have to include, then, the prevention of the Ca^{2+} accumulation by these organelles.

Disturbances in membrane function and in the plasma membrane in particular also characterize the loss of reversibility in ischemic injury. This has led to the other major hypothesis to explain this loss.[15] Defective cell membrane function is an early feature of irreversible ischemic injury and may be the primary event in the genesis of the irreversible state.[15] The case for alterations in membrane function in general and in the plasma membrane in particular as the cause of irreversible injury in ischemia is quite strong. Results of morphologic, functional, and biochemical studies strongly suggest that defects in cell membranes are an early feature of irreversible myocardial cell injury.[12,16-27]

Two specific functional consequences of membrane damage in irreversibly injured cells have been related specifically to the loss of viability. Defects in cell volume regulation have been described and are characterized by an increased inulin-diffusible space, increased tissue H_2O and Na^{2+} levels (for a review see Jennings et al[15]). While it cannot be denied that these changes could affect cell viability, there is, in fact, very little direct evidence that they actually do. On the contrary, the evidence suggests that such disturbances in Na^+ and K^+ contents are not necessarily lethal.[28]

A much better case can be made for the changes in calcium homeostasis as causally related to the loss of cell viability in ischemia. Calcium ions are biologically quite active and when present in excess are capable of considerable disruption of metabolic order. Recent evidence from our laboratory has implicated Ca^{2+} ions in the causation of toxic cell death.[29-31] It would seem reasonable to suspect that they act similarly in ischemic cell injury.

These considerations emphasize several unanswered questions bearing on the pathogenesis of the irreversible injury in ischemia. With respect to the mitochondria, what factors are responsible for the progressive loss of inner membrane function? What is the biochemical nature of the alteration? What role do Ca^{2+} ions play in the inability to restore mitochondrial function upon the reperfusion of irreversibly injured cells? Similarly, with respect to the dysfunction of other cellular membranes, what is the biochemical basis for these alterations? Are they indeed the basis for the loss of reversibility of the cell injury in ischemia?

Experimental Liver Ischemia

Studies in our laboratory over the last 4 years using *in situ* liver and myocardial ischemia have sought answers to these questions. We have been able to assess the role of the mitochondrion in the irreversibility of ischemic cell injury and have reached some understanding of the cause and biochemical nature of the changes in this organelle. The biochemical basis of the dysfunction of the other cellular membranes also has been identified. We have gained some insight into the relationship between this biochemical alteration and their functional manifestations. Finally, we have

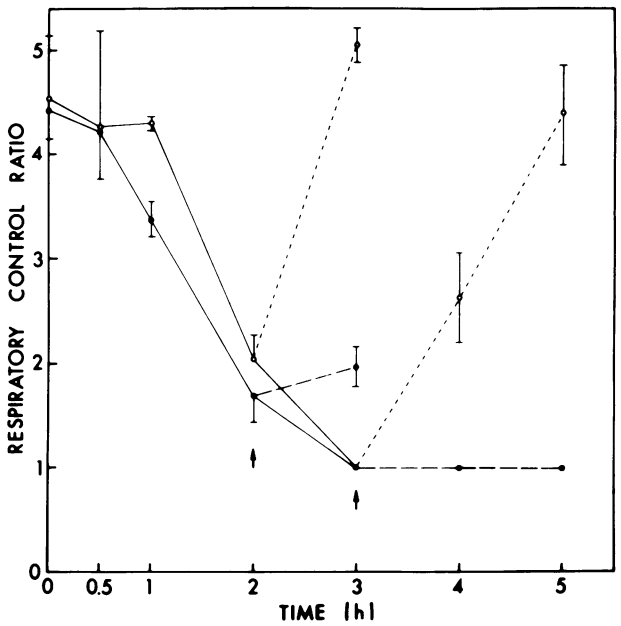

Figure 1—Respiratory control in ischemic liver mitochondria. Liver tissue in the intact rat was made ischemic for the times indicated either with (*open circles*) or without (*closed circles*) chlorpromazine pretreatment (30 mg/kg body weight). After either 2 or 3 hours of ischemia (as indicated by the *arrows*), the livers were reperfused *in vivo* by the removal of the arterial clamp occluding the arterial and portal blood supply to the left lateral and median lobes; ----, mitochondria prepared subsequent to reperfusion; ——, mitochondria prepared from ischemic livers without reperfusion. Mitochondria were isolated, and the respiratory control ratio was measured as described.[8] The reaction mixture contained 0.225 M sucrose, 10 mM potassium phosphate (pH 7.4), 5 mM $MgCl_2$, 20 mM KCl, and 20 mM triethanolamine buffer, pH 7.4. The oxidizable substrate was 3.3 mM glutamate-malate. After equilibration of the medium at 25 C, mitochondria (2–4 mg protein) were added and the rate of O_2 consumption measured. Stage 3 respiration was initiated by adding 450 nmol of ADP. The results are the mean ± standard deviation of separate preparations from each of three animals. Reprinted with permission.[34]

begun to explore the very early events that may trigger the whole process.

Ischemic liver tissue is produced by clamping the portal venous and hepatic arterial blood supply to the left lateral and median lobes of a rat liver.[13,32,33] Total occlusion of the portal vein and hepatic artery results in splanchnic pooling of the blood and subsequent death of the animal. If after 0.5 hour of ischemia reflow to the liver is established by operating on the animal a second time and removing the clamp, there is little or no evidence of liver cell death. The cells maintain their ability to regenerate ATP levels, which had fallen to 10% of the control, and show no detectable increase in total or mitochondrial calcium levels upon reflow.[13] In contrast, livers subjected to 2–3 hours of ischemia reveal extensive necrosis when examined 24 hours after reflow.[13] They are incapable of regenerating the ischemia-induced fall in ATP.[13] Evidence of membrane dysfunction[32,33] and altered calcium homeostasis is found. There is a 3–4-fold increase in tissue and mitochondrial calcium following reflow.[13] Since the liver cells, then, are relatively able to withstand the effects of 0.5 hour of ischemia followed by the immediate restoration of blood flow, they can at this time be categorized as reversibly injured. They still maintain the critical function(s) required for the survival of the cell lost during more prolonged periods of ischemia. Liver cells subjected to 2–3 hours of ischemia—a time period that produces marked liver cell death after reestablishment of blood flow—are irreversibly injured and, therefore, have lost this critical cellular function.

Liver cells can be protected from the effect of otherwise irreversible periods of ischemia by pretreatment with chlorpromazine.[13] Pretreatment with chlorpromazine, 30 minutes before inducing ischemia for up to 3 hours, completely prevented the cell death associated with this duration of ischemia. This protective action of chlorpromazine was confirmed by the ability of the treated animals to regenerate cellular ATP levels after 3 hours of ischemia. In addition, chlorpromazine was shown to significantly reduce the increase in total liver cell and mitochondrial Ca^{2+} contents that accompany the return of blood flow to irreversibly injured liver cells[13] (see Table 2). The action of chlorpromazine could not be attributed to any effect on the rate or extent to which the liver cells became ischemic or on the perfusion patterns following release of the obstruction (there is no "no-reflow" phenomenon). It was concluded that the action of chlorpromazine must be on some component(s) of the reaction of the cells to the ischemia itself.

Role of Mitochondria in Irreversible Ischemic Injury

The ability of chlorpromazine to prevent ischemic liver cell death was used to evaluate the role of mitochondrial alterations in the genesis of irreversible injury.[34] Mitochondria were isolated from ischemic livers and were assayed for respiratory control with glutamate/malate as substrate. Figure 1 indicates that such mitochondria showed a progressive loss of respiratory control, the respiratory control ratio (RCR) falling to the theoretic limit of 1 with 3 hours of ischemia. After 2 or 3 hours of ischemia, the livers were reperfused with arterial blood by reoperation and removal of the arterial clamp. After 1 and 2 hours mitochondria were isolated and assayed. There was essentially no recovery of respiratory control. This is consistent with the fact that the liver cells were irreversibly injured after such ischemic periods, and it is such a correlation between the loss of reversibility and the inability to reverse mitochondrial dys-

function that has led to the suggestion that the two are causally related.

However, if the rats were pretreated with chlorpromazine and then liver ischemia induced for the same times, there was a similar loss of respiratory control (Figure 1, open circles). With chlorpromazine pretreatment, the RCR still fell to 1 with 3 hours of ischemia. In this case, however, reperfusion of the livers after either 2 or 3 hours of ischemia resulted in complete recovery of respiratory control (broken lines in Figure 1).

This experiment suggests that the loss of mitochondrial function with ischemia is reversible. The inability to restore mitochondrial function during reperfusion in the absence of chlorpromazine would then be the metabolic consequence of reperfusion itself. Consequently, mitochondrial alterations could not be the cause of the irreversibility of the cellular deterioration and death during the reperfusion period. The validity of this conclusion is dependent upon there being no effect of chlorpromazine on the reaction of the mitochondria to ischemia. To date we have detected 15 alterations in mitochondrial structure and function in ischemic liver cells (Table 1).[34,35] With each of these, there was no difference between mitochondria isolated from ischemic livers either with or without chlorpromazine pretreatment.[34,35] We can conclude, then, that chlorpromazine does not affect the reaction of liver mitochondria to ischemia. The lack of restoration of mitochondrial function during reperfusion in animals not pretreated with chlorpromazine, therefore, cannot be the consequence of any known mitochondrial alteration. In addition, these mitochondrial alterations cannot be the cause of the irreversibility of the cellular deterioration and death during the reperfusion period.

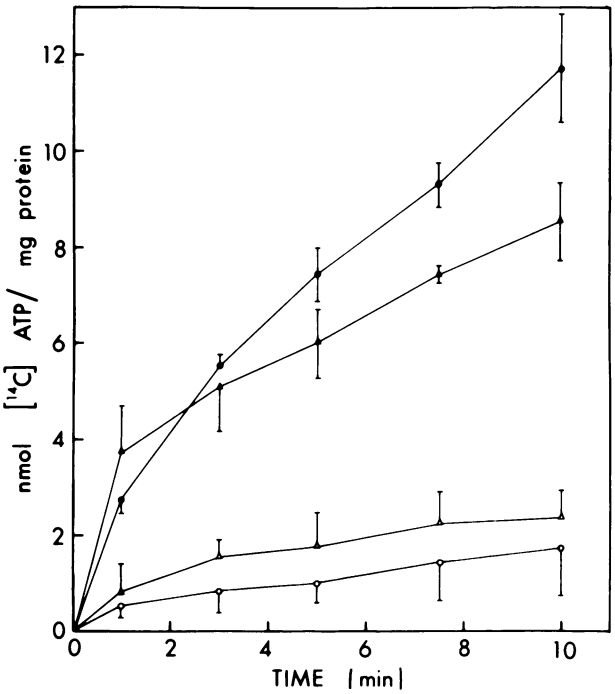

Figure 2—Adenine nucleotide translocase activity in ischemic liver mitochondria. Mitochondria were prepared from sham operated livers (*closed circles*); livers ischemic for 3 hours (*open circles*); liver ischemic for 3 hours in chlorpromazine (30 mg/kg body weight)-pretreated animals (*open triangles*); and liver ischemic for 3 hours and then reperfused *in vivo* for 2 hours in chlorpromazine-treated animals (*closed triangles*). Adenine nucleotide translocase was assayed by adding approximately 1 mg of protein to 1 ml of a solution containing 110 mM KCl, pH 7.4, 0.2 mM EDTA, and 70 nmol of [^{14}C] ATP (3 to 5 × 10^5 cpm). After incubation at 4 C, the reaction was stopped by the addition of 50 μmol atractyloside. The samples were centrifuged within 2 minutes of the addition of atractyloside for 10 minutes at 10,000*g*. The supernatant was discarded and the pellet dissolved in 150 μl 0.5% SDS. A 100-μl aliquot of the dissolved mitochondrial pellet was counted in 10 ml Aquasol. The results are the mean ± SD of separate preparations from each of 3 animals. Reprinted with permission.[34]

Table 1—Ischemic Mitochondrial Alterations That Develop With or Without Chlorpromazine

1. Complete loss of respiratory control
2. Decreased ADP:O ratio when RCR greater than 1
3. Decreased contents of cytochrome aa_3 and cytochrome c + c_1
4. Loss of DNP-stimulated O_2 uptake
5. Altered ATPase:
   a. increased basal activity (No Mg^{2+} or DNP)
   b. decreased DNP-stimulated activity
6. Loss of adenine nucleotide translocase activity
7. Reduction in one protein band of Mr = 83,000
8. Large amplitude swelling
9. Amorphous matrix densities
10. Failure to generate membrane potential
11. Loss of valinomycin-stimulated O_2 uptake
12. Decreased K^+ content
13. Decreased Mg^{2+} content
14. Increased Na^+ content
15. Increased inner membrane permeability

In each case, the structural or functional alteration refers to the effect of 3 hours of liver ischemia either with or without chlorpromazine pretreatment (30 mg/kg body weight).

We currently suspect that there are 2 fundamental changes related to the structure and function of the inner mitochondrial membrane affected by ischemia and relating, in turn, to most of the changes in Table 1. The first of these is a loss of adenine nucleotide translocase activity, reported initially in ischemia heart muscle.[36-38] Figure 2 shows that 3 hours of ischemia produced a very significant loss of adenine nucleotide translocase in ischemic liver mitochondria.[34] A similar loss occurred in the mitochondria from 3 animals ischemic for 3 hours that were pretreated with chlorpromazine.[34] Recovery of adenine nucleotide translocase activity with blood reflow following 3 hours of ischemia was achieved in animals pretreated with chlorpromazine. There was no recovery in the absence of chlorpromazine pretreatment.[34]

Long chain fatty acyl-CoA esters have been shown to be capable of reversibly inhibiting adenine nucleotide translocase when added *in vitro* to liver or heart mitochondria.[39-43] A positive correlation has been reported in liver between the tissue concentration of long chain acyl-CoA esters and inhibited translocation.[48] Tissue levels of fatty acyl-CoA are normally low but increase during ischemia.[44-46] Loss of adenine nucleotide translocase, therefore, probably results from the inhibitory effect of the accumulation of these long chain acyl-CoA esters. The presence of adenosine 3'-pyrophosphate in the acyl-CoA molecule may account for this inhibition.[38] Loss of adenine nucleotide translocase, however, can only explain some of the mitochondrial dysfunction, not all.[35]

A second alteration contributing to the spectrum of ischemic mitochondrial alterations is an increase in the permeability of the inner membrane.[35] With or without chlorpromazine pretreatment, mitochondria isolated from ischemic liver will not generate a membrane potential; they will respond neither to dinitrophenol nor to valinomycin with an increased 0_2 consumption; they have lost internal K^+ and Mg^{2+} and have gained Na^2 ions; and they exhibit high amplitude osmotic swelling.[35] These changes are easily explained by an increase in the permeability of the inner membrane. Such an increased permeability was demonstrated by measuring the extent to which mitochondria will shrink under the influence of isomolar polyethylene glycols of different molecular weights.[35]

Similar alterations can be induced *in vitro*. Low levels of calcium will induce a reversible increase in the permeability of the mitochondrial inner membrane accompanied by collapse of the membrane potential and by the onset of large amplitude swelling.[47-51] Endogenous Ca^{2+} and Mg^{2+} contents are equilibrated with the medium, since both neutral and charged molecules of molecular weight less than 1000 readily pass through the inner membrane. An identical membrane change can be induced by diamide, inorganic phosphate, arsenate, fatty acids, and, most interestingly, palmitoyl coenzyme A.[52] These agents seem to act as potentiators of the inducing activity of Ca^{2+} ions. Recent studies in our laboratory indicate that the metabolic alterations induced by very low levels of exogenous calcium in the presence of palmitoyl-CoA reproduce many of the features of the effect of ischemia on mitochondrial function *in vivo*.[35]

The molecular mechanism responsible for the change in the inner membrane is not resolved. One hypothesis maintains that this membrane contains units which on binding Ca^{2+} are able to open a transmembrane hydrophilic channel.[49] Alternatively, it is suggested that a very limited phospholipid degradation is sufficient to produce a modification of the inner membrane permeability properties.[51]

Table 2—Effect of Chlorpromazine on the Calcium of Mitochondria Isolated From Ischemic Rat Liver Cells[13]

Treatment	Mitochondrial calcium content (nmoles Ca^{2+}/kg protein)
Sham	3.4 ± 0.3
3 Hours ischemia—no-reflow	3.6 ± 0.6
3 Hours ischemia—1 hour reflow	13.7 ± 2.7
Pretreatment with chlorpromazine—3 hours ischemia—1 hour reflow	3.9 ± 0.3

Values are the mean ± SD of the calcium content of mitochondria prepared from 3 separate animals.

In either case, it seems likely that high levels of long chain acyl-CoA esters are responsible for many of the pathologic mitochondrial changes in ischemia. These mitochondrial alterations are all reversible. However, the biochemical environment created by the reperfusion of cells irreversibly injured by unrelated mechanisms prevents the restoration of normal mitochondrial structure and function. We suggest that it is the large influx of Ca^{2+} ions upon reperfusion that prevents restoration of mitochondrial function. Table 2 indicates the changes in liver mitochondrial Ca^{2+} content with ischemia and reperfusion in the presence and absence of chlorpromazine. With chlorpromazine, there is no accumulation of calcium upon reperfusion, and mitochondrial function can be restored.

The alteration(s) responsible for the large increases in total cell and mitochondrial calcium content upon reperfusion is probably the critical lesion underlying the irreversibility of ischemic injury. Cellular membranes play a central role in Ca^{2+} homeostasis both as a permeability barrier to the passive diffusion into the cell down a very steep concentration gradient and as the site of active efflux against this same gradient. Phospholipids are a major component of these membranes, and ischemia induces a significant alteration in phospholipid metabolism.

Membrane Alterations Accompanying Irreversible Ischemia

We have shown that both rat liver and heart *in situ* ischemia produce a progressive loss of phospholipids from cellular membranes.[33,53] Whole homogenates and postmitochondrial supernatants from livers ischemic for 3 hours showed a 40% and 55% decrease in phospholipids, respectively (Figures 3 and 4). Phosphatidylcholine and phosphatidylethanolamine were

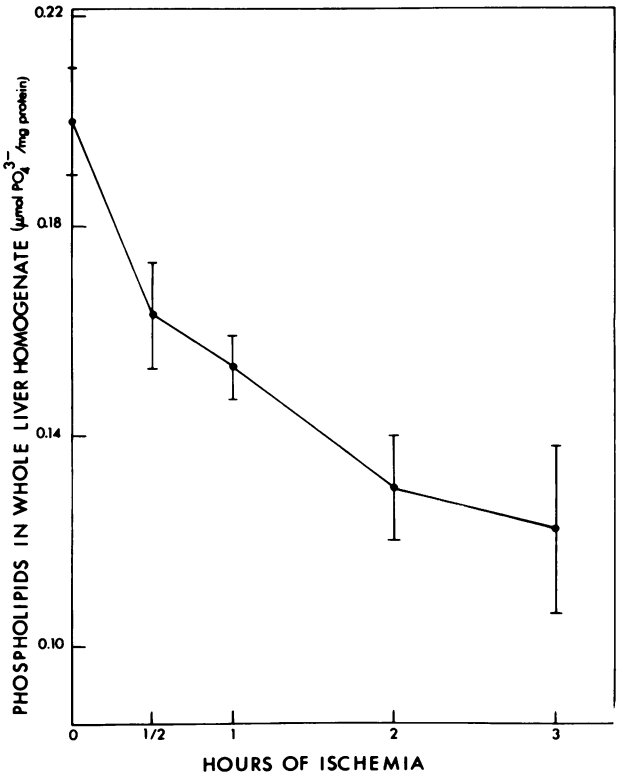

Figure 3—Phospholipid content of whole homogenates of ischemic liver tissue. Rat liver tissue was made ischemic for the times indicated. Phospholipids in whole homogenates of this tissue were extracted by modification of the method of Bligh and Dyer.[33] Phosphate was measured on dried aliquots of the lipids. The total protein content of the whole homogenates was not altered, while there was a slight increase in the weights of the ischemic tissue.[33] Results are the mean ± SD of separate determinations on 3 animals. Reprinted with permission.[33]

predominantly affected without accumulation of either of the corresponding lysophospholipids. Pretreatment of the animals with chlorpromazine prevented the loss of phospholipids from both the whole homogenates and postmitochondrial supernatants.[33] This phospholipid depletion was entirely accountable by an accelerated rate of degradation with a halflife of 2–4 hours for ischemia, as compared with 24 hours for control microsomal membrane phospholipids.[33]

Rat myocardial cells react to lethal doses of ischemia in a very similar manner. An accelerated degradation of phospholipid preceded the loss of viability of the major portion of free-wall myocardium, as assessed by morphologic changes and an increased Ca^{2+} content subsequent to ligation of the left coronary artery of an adult rat heart.[31] As in the liver, pretreatment with chlorpromazine prevented both this alteration in phospholipid metabolism and the necrosis of the free-wall myocardium.[53]

The accelerated degradation of phospholipids produced by ischemia is associated with considerable membrane dysfunction,[32,33,53] We have made use of liver cell microsomal membranes and heart cell sarcoplasmic reticulum as models of the effect of ischemia on cellular membranes. Hepatic microsomes prepared from the lipid-depleted postmitochondrial supernatants exhibited considerable alterations in their structure and function with inhibition of a glucose-6-phosphatase[32] and calcium pump activities[32] and a 25–50-fold increase in their passive permeability to Ca^{2+}.[33] Pretreatment with chlorpromazine prevented this increased Ca^{2+} permeability, and addition of phosphatidylcholine to the ischemic microsomes restored a normal Ca^{2+} permeability.[33] Electron micrographs of freeze-fractured ischemic microsomes showed fewer intramembranous particles, bare membrane regions devoid of particles, and areas containing aggregations of intramembranous particles.[33]

Sarcoplasmic reticulum isolated from ischemic myocardial cells showed a time-dependent loss of phospholipid with a parallel loss of active Ca^{2+} uptake that reached 60% with a total lipid depletion from these membranes of 33%.[53] An increased passive permeability to Ca^{2+} also was demonstrated. Chlorpromazine protected against the loss of phospholipids, the inhibition of Ca^{2+} uptake, and the in-

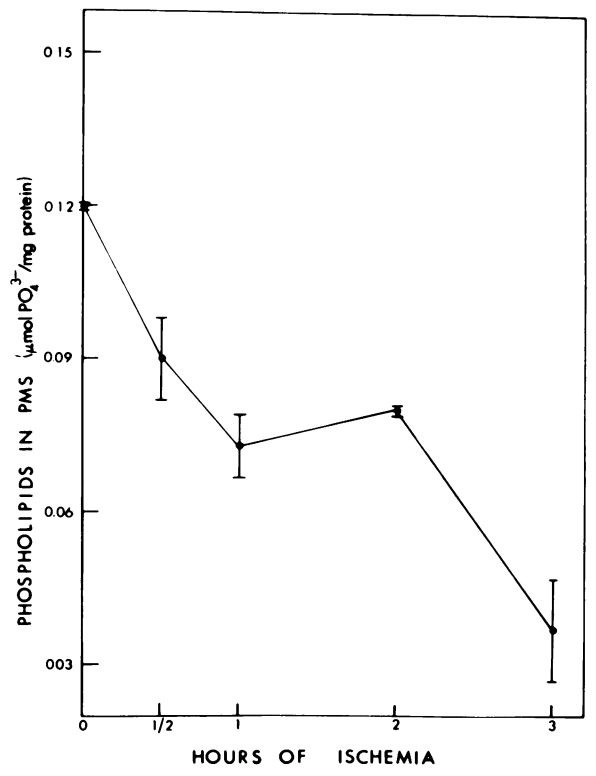

Figure 4—Phospholipid content of postmitochondrial supernatants derived from ischemic liver tissue. Rat liver tissue was made ischemic for the times indicated. Post mitochondrial supernatants (10,000*g* supernatant) of the ischemic tissue were prepared and the phospholipid content determined as described in the legend to Figure 3. Results are the mean ± SD of separate determinations on 3 animals. Reprinted with permission.[33]

creased Ca^{2+} permeability of the sarcoplasmic reticulum.[53]

A number of reasons lead us to believe that this disturbance in phospholipid metabolism is the critical alteration that produces irreversible cell injury in ischemia. In both the liver and the heart, the time course closely parallels that of the loss of reversibility. Chlorpromazine prevents the changes in phospholipid in parallel with the prevention of cell death. Finally, the functional consequences, particularly as reflected in an altered permeability of isolated microsomes and sarcoplasmic reticulum to Ca^{2+} ions, are relevant to the increasing evidence that disturbed membrane function with changes in intracellular Ca^{2+} homeostasis participate in the production of irreversible ischemic cell damage.

The critical link in any such sequence connecting an accelerated phospholipid degradation with the onset of irreversible injury is the plasma membrane, for the changes in cell physiology that characterize irreversibly injured ischemic cells are specifically indicative of plasma membrane dysfunction. Attempts to isolate plasma membranes from ischemic liver cells by the conventional methods based on membrane buoyant density have been unsuccessful. However, with freeze-fracture electron microscopy there is in ischemic rat liver plasma membranes an aggregation of the membrane-associated particles dependent on the duration of ischemia and prevented by pretreatment with chlorpromazine.[54] This suggests that alterations in the structure of the plasma membrane occur in parallel with the previously described changes in microsomal membrane structure. Similar changes have also been reported in ischemic kidney and heart muscle plasma membrane.[23,24]

Two aspects of this disorder in phospholipid metabolism produced by ischemia currently occupy our attention. First, what is the molecular basis in the structural organization of ischemic membranes for the increased permeability? Second, what is the cause of the accelerated degradation of membrane phospholipids that results in this structural alteration?

A clue to the answer to the first question came from the freeze-fracture ultrastructure of ischemic liver cell membranes.[54,56] With this technique ischemic liver cell microsomal and plasma membranes appear very similar to membranes examined at reduced temperatures. In both cases, there is aggregation of the intramembranous particles in the lateral plane of the membrane with the appearance of particle-free bare areas of varying size. Changes in the distribution of membrane-associated particles at low temperature have been attributed to phase transitions (liquid crystal to gel state) in the membrane phospholipid bilayer.[55-63] The lateral motion and consequent aggregation of intramembranous particles with reduced temperatures is attributed to the growth of protein-excluding regions of liquid crystal to gel state phase transitions in the bilayer lipid that are observed as the smooth, particle-free patches.

By wide-angle X-ray diffraction, we have recently demonstrated the presence of similar gel phase phospholipid in microsomal membranes isolated from rat liver ischemic for 3 hours.[64] Gel phase lipid could not be demonstrated in the controls. The presence of gel and liquid crystalline lipid in the plane of the membrane, ie, lateral phase separations, can be directly related to the increased permeability. It is known that the permeability of liposomes towards various solutes drastically increases at the phase transition of the lipid.[65-68] At this critical temperature, in these liposomes gel and liquid crystalline lipid coexist, as in the ischemic membranes. The interfaces between the separate phases is believed to be the site of the increased permeability of such bilayers. It is our working hypothesis that the progressive depletion of phospholipid from cellular membranes in ischemia alters the equilibrium between the membrane proteins and the remaining lipid such that a new state is created, characterized by the appearance of gel phase phospholipid and the aggregation of the proteins into the remaining liquid crystalline lipid. We suspect that the boundaries between such laterally separated phases represent sites of increased membrane permeability.

The similarity between the disturbance in phospholipid metabolism induced by ischemia in rat myocardial and liver cells suggests that they have a common pathogenesis. Currently there are, at least, three mechanisms that appear as likely causes of the accelerated phospholipid degradation in ischemic cells. The first is the accumulation of potentially damaging concentrations of normal metabolic intermediates. The recently reported high levels of both long-chain acyl-CoA esters and long-chain acyl carnitine in ischemic cells[44-46] suggest that these intermediates may have generalized detergent effects that could disrupt membrane structure. Palmitylcarnitine *in vitro* produced marked changes in the structure and function of cardiac sarcoplasmic reticulum and sarcolemmal Na^+, K^+-ATPase isolated from canine ventricular muscle.[69] Palmitylcarnitine was proposed as "a naturally occurring detergent."[69] Although it is not possible at this time to ascribe any *in vivo* alterations in membrane structure and function to this mechanism, the elevated levels of palmitylcarnitine that occur during myocardial ischemia[44-46] could act to alter membrane permeability.

Release of lysosomal enzymes is a potential alternative to the detergent action of the long-chain acyl carnitine as a mechanism responsible for the acceler-

ated phospholipid degradation. There have been several reports of biochemical alterations in cardiac lysosomes subsequent to coronary occlusion,[70-76] and the potential certainly exists for activation of multiple lysosomal enzymes early in ischemia. Direct evidence, however, that any of these enzymes actually contributes to the cellular damage is not available. The problem is really twofold. Is there intracellular release of lysosomal enzymes prior to the onset of irreversible injury? If so, do these enzymes contribute to the cellular injury?

With regard to the first of these questions, studies of hypoxic fetal mouse hearts maintained in organ culture suggested that lysosomal enzyme changes with this ischemic-like stress are minimal in the injury phase.[77] Evidence of activation of lysosomal enzyme function was evident during the recovery period accompanying reoxygenation. Similarly, in an hypoxic, isolated perfused heart system, marked enzyme release could not be demonstrated until irreversible injury had already occurred.[78] On the other hand, biochemical redistribution of cathepsin D was evident, while the injury was still sublethal. This early biochemical shift was felt to reflect increased fragility of the lysosomes during homogenization of the tissues, rather than an actual translocation of hydrolases *in vivo*.[79]

With respect to the consequences of lysosomal enzyme redistribution, breakdown of cellular components as a result of the release of lysosomal enzymes is hypothesized to produce irreversible cell injury in a number of pathologic states. Release of lysosomal enzymes into the cytoplasm follows phagocytosis of urate crystals[80] or silica particles.[81,82] by polymorphonuclear leukocytes or macrophages. A similar mechanism is postulated to cause cell death following exposure to heavy metals,[83] photosensitizing dyes,[84] and viruses,[85] as well as ischemia. Although intracellular lysosomal rupture has been documented in these other systems, the evidence that it causes cell death is only circumstantial. Recent studies of the silica-induced killing of macrophages showed no evidence that the intracellular rupture of lysosomes was associated with any measurable degradation of cellular macromolecules, including phospholipids.[86]

While it is still premature to make any firm conclusions, it would seem that accumulating evidence from a number of different sources makes it unlikely that the release of lysosomal enzymes into the cytoplasm contributes to the accelerated degradation of phospholipid during the development of irreversible ischemic injury.

The third mechanism and the one we currently favor to explain accelerated lipid degradation is the activation of endogenous, membrane-bound phospholipases by a redistribution of cellular Ca^{2+} ions. Phospholipase A is an almost universal activity of cells.[87] The role of these enzymes in normal cellular physiology has not been clearly defined, although it seems likely that they participate in the regular turnover of fatty acids in membrane phospholipids. While the subcellular localization of phospholipases has been examined in a number of different tissues, rat liver is probably the best characterized. Rat liver mitochondria contain a Ca^{2+}-dependent phospholipase A_2 with an alkaline pH optimum.[88,89] Rat liver microsomes[89,90] and plasma membranes[89,91] also contain phospholipase A activity. These extramitochondrial enzymes all have alkaline pH optima and require Ca^{2+}. Phospholipase A activity has similarly been demonstrated in rat heart "microsomes" (sarcoplasmic reticulum).[92] Phosphatidylethanolamine was preferred to phosphatdylcholine as the substrate, similar to the substrate specificity of the liver enzymes.[93]

The endogenous lipid degrading system in the membranes of rat liver endoplasmic reticulum is capable upon Ca^{2+} activation of considerable disruption of normal structure and function. These changes bear a close resemblance to those which occur in the same membranes *in vivo* with ischemia.[94] Phosphatidylethanolamine and to a lesser extent phosphatidylcholine are degraded to methanol-water soluble products. The depletion of microsomal membrane phospholipid is accompanied by a loss of glucose-6-phosphatase and of cytochrome P-450. Polyacrylamide gel electrophoresis revealed no differences between the pattern or relative amounts of the solubilized membrane proteins before or after depletion of membrane phospholipid.

There is increasing evidence that changes in intracellular Ca^{2+} homeostasis are a very early feature of the effect of the ischemia on cells (for a review see Naylor et al[95]). The rise in resting tension in hypoxic heart muscle with no change in the total Ca^{2+} content suggests a redistribution of intracellular Ca^{2+} ions with a raised cytosolic concentration. We would suggest that such an elevated cytosolic Ca^{2+} could result in activation of membrane phospholipases.

Recapitulation

Irreversible ischemic cell injury is characterized by dysfunction of cellular membranes. Previous studies suggested that the effects of such alterations on mitochondrial or plasma membranes are related to the loss of reversibility of the cell injury in ischemia. The investigations in our laboratory reviewed above have

thrown some light on the cause of the membrane injury in each of these organelles and have allowed some conclusions with regard to the role that each plays in the irreversible cell injury in ischemia.

Mitochondrial function is reversibly disrupted. A number of structural and functional alterations can be accounted for, in large part, by inhibition of adenine nucleotide translocation and an increase in the permeability of the inner mitochondrial membrane. Each of these alterations seems, in turn, to be a consequence of the metabolic changes produced by ischemia. In ischemic cells, β-oxidation of fatty acids is rapidly inhibited,[89,90] and there is a concomitant increase in tissue metabolite intermediates, particularly long-chain acyl-CoA esters.[46-48] Mitochondrial function can be restored upon reoxygenation as the cells recover the capacity to oxidize fatty acids, provided that the functional consequences of the disruption of plasma membrane structure are prevented, as with chlorpromazine pretreatment.

A very different biochemical lesion characterizes the effect of ischemia on other cellular membranes. In both liver and heart cells, ischemia results in an accelerated degradation of phospholipids from the membranes of the endoplasmic and sarcoplasmic reticulum, respectively, and probably also from the plasma membranes. In this case, the relevant functional consequence of this phospholipid degradation is a markedly increased permeability of these membranes to Ca^{2+} ions. All cells in the body are bathed in a fluid very rich in Ca^{2+} ions (10^{-3} M), while intracellular Ca^{2+} concentrations are much lower, on the order of 10^{-7} to 10^{-6} M. The electrical potential across the plasma membrane of these cells tends to drive Ca^{2+} into them. Such a large electrochemical gradient is maintained by the relative impermeability of the plasma membrane to Ca^{2+} and by active extrusion. Damage to the plasma membrane with disruption of this permeability barrier allows influx of Ca^{2+} ions. We suspect that this accelerated phospholipid degradation is a consequence of the activation of endogenous phospholipases as a result of a disturbance in calcium homeostasis that is not related to increased influx, in this case a redistribution of intracellular Ca^{2+} ions.

In conclusion, we are arguing for a very central role for calcium ions in the pathogenesis of ischemic cell injury. These ions not only initiate the biochemical alteration that is responsible for irreversible cell injury, but they are also the mediators, in turn, of the functional consequences of this injury. The influx of calcium ions prevents restoration of mitochondrial function lost, in part, as a result of a change in the inner membrane initiated again by calcium ions potentiated by long-chain acyl-CoA esters. These conclusions are clearly based on normal cellular physiology and the expected alterations in these functions that result from ischemia. With both types of membrane injury that we have discussed there is a relationship between normal metabolic functions and the development of membrane damage. This point is important, for it is clear that cells can only react in the ways that their programmed, biochemical organization allows. There is really no such thing as a "pathologic reaction." It is encouraging that our understanding of one of the most important cellular alterations in pathology, irreversible ischemic cell injury, is progressing to the point where this conclusion clearly applies.

References

1. Ames A III, Wright RL, Kowada M, Thurston JM, Majno G: Cerebral ischemia: II. The no-reflow phenomenon. Am J Pathol 1968, 52:437-454
2. Leaf A: Regulation of intracellular fluid volume and disease. Am J Med 1970, 49:291-295
3. Summers WK, Jamison RL: The no-reflow phenomenon in renal ischemia. Lab Invest 1971, 25:635-643
4. Willms-Kretschmer K, Majno G: Ischemia of the skin. Electron microscopic study of vascular injury. Am J Pathol 1969, 54:327-354
5. Trump BF, Mergner WJ, Kahng MW, Saladino AJ: Studies on the subcellular pathophysiology of ischemia. Circulation 1976, 53:I-17-I-26
6. Jennings RB: Discussion: Relationship of acute ischemia to functional defects and irreversibility. Circulation 1976, 53:I-26-I-29
7. Vogt MT, Farber E: On the molecular pathology of ischemic renal cell death: Reversible and irreversible cellular and mitochondrial metabolic alterations. Am J Pathol 1968, 53:1-26
8. Gaja G, Ferrero ME, Piccoletti R, Bernelli-Zazzera A: Phosphorylation and redox states in ischemic liver. Exp Mol Pathol 1973, 19:248-265
9. Laiho KU, Trump BF: The relationship between cell viability and changes in mitochondrial ultrastructure, cellular ATP, ion and water content following injury of Ehrlich ascites tumor cells. Virchows Arch [Cell Pathol] 1974, 15:267-277
10. Trump BF, Laiho KU: Studies of cellular recovery from injury: 1. Recovery from anoxia in Ehrlich ascites tumor cells. Lab Invest 1975, 33:706-711
11. Mergner WJ, Smith MW, Trump BF: Studies on the pathogenesis of ischemic cell injury: XI. P/O Ratio and acceptor control. Virchows Arch [Cell Pathol] 1977, 26:17-26
12. Whalen DA Jr, Hamilton DG, Ganote CE, Jennings RB: Effect of a transient period of ischemia on myocardial cells: 1. Effects on cell volume regulation. Am J Pathol 1974, 74:381-398
13. Chien KR, Abrams J, Pfau RG, Farber JL: Prevention by chlorpromazine of ischemic liver cell death. Am J Pathol 1977, 88:539-558
14. Greenawalt JW, Rossi CS, Lehninger AL: Effect of active accumulation of calcium and phosphate ions on the structure of rat liver mitochondria. J Cell Biol 1964, 23:21-38
15. Jennings RB, Ganote CE, Reimer K: Ischemic tissue injury. Am J Pathol 1975, 81:179-198

16. Macknight ADC, Leaf A: Regulation of cellular volume. Physiol Rev 1977, 57:510–573
17. Jennings RB, Sommers HM, Kaltenbach JP, West JJ: Electrolyte alterations in acute myocardial ischemic injury. Circ Res 1964, 14:260–269
18. Shen AC, Jennings RB: Myocardial calcium and magnesium in acute ischemic injury. Am J Pathol 1972, 67: 417–440
19. Flores J, DiBona DR, Beck CH, Leaf A: The role of cell swelling in ischemic renal damage and the protective effect of hypertonic solute. J Clin Invest 1972, 51: 118–126
20. Flores J, DiBona DR, Frega N, Leaf A: Cell volume regulation and ischemic tissue damage. J Membr Biol 1972, 10:331–343
21. Leaf A: Cell swelling (editorial) A factor in ischemic tissue injury. Circulation 1973, 48:455–458
22. 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
23. Coleman SE, Duggan J, Hackett RL: Plasma membrane changes in freeze-fractured rat kidney cortex following renal ischemia. Lab Invest 1976, 35:63–70
24. Ashraf M, Halverson CA: Structural changes in the freeze-fractured sarcolemma of ischemic myocardium. Am J Pathol 1977, 88:583–594
25. Beller GA, Conroy J, Smith TW: Ischemia-induced alterations in mycocardial ($Na^+ + K^+$)-ATPase and cardiac glycoside binding. J Clin Invest 1976, 57: 341–350
26. Jennings RB, Ganote CE, Kloner RA, Whalen DA Jr, Hamilton DG: Explosive swelling of myocardial cells irreversibly injured by transient ischemia. Recent Advances in Studies on Cardiac Structure and Metabolism. Edited by A Fleckenstein, G Rona. Vol 6. Baltimore, University Park Press, 1975, pp 405–413
27. Burton KP, Hagler HK, Templeton GH, Willerson JT, Buja LM: Lanthanum probe studies of cellular pathophysiology induced by hypoxia in isolated cardiac muscle. J Clin Invest 1977, 60:1289–1302
28. Judah JD, Ahmed K, McLean AEM, Christie GS: Ion transport in ethionine intoxication, Biochemical Pathology. Edited by E Farber, PN Magee. Baltimore, Williams and Wilkins, 1966, pp 167–175
29. Schanne FAX, Kane AB, Young EE, Farber JL: Calcium dependence of toxic cell death: A final common pathway. Science 1979, 206:700–702
30. Kane AB, Young EE, Schanne FAX, Farber JL: Calcium dependence of phalloidin-induced liver cell death. Proc Natl Acad Sci USA 1980, 77:1177–1180
31. Schanne FAX, Pfau RG, Farber JL: Galactosamine-induced cell death in primary cultures of rat hepatocytes. Am J Pathol 1980, 100:25–36
32. Chien KR, Farber JL: Microsomal membrane dysfunction in ischemic rat liver cells. Arch Biochem Biophys 1977, 180:191–198
33. Chien KR, Abrams J, Serroni A, Martin JT, Farber JL: Accelerated phospholipid degradation and associated membrane dysfunction in irreversible, ischemic liver cell injury. J Biol Chem 1978, 253:4809–4817
34. Mittnacht S Jr, Sherman SC, Farber JL: Reversal of ischemic mitochondrial dysfunction. J Biol Chem 1979, 254:9871–9878
35. Mittnacht S Jr, Farber JL: Unpublished observations
36. Shug AL, Shrago E, Bittar N, Folts JD, Koke JR: Acyl-CoA inhibition of adenine nucleotide translocation in ischemic myocardium. Am J Physiol 1975, 228: 689–692
37. Shug AL, Koke JR, Folts JD, Bittar N: Recent Advances in Studies on Cardiac Structure and Metabolism. Edited by RE Roy and G Rona. Vol 10. Baltimore, University Park Press, 1975, pp 365–378
38. Shrago E, Shug AL, Bittar N, Folts JD: Control of energy production in myocardial ischemia. Circ Res 1976, 38(Suppl. 1):75–78
39. Pande SV, Blanchaer MC: Reversible inhibition of mitochondrial adenosine diphosphate phosphorylation by long chain acyl coenzyme A esters. J Biol Chem 1971, 246:402–411
40. Shug A, Lerner E, Elson C, Shrago E: The inhibition of adenine nucleotide translocase activity by oleoyl CoA and its reversal in rat liver mitochondria. Biochem Biophys Res Commun 1971, 43:557–563
41. Harris RA, Farmer B, Ozawa T: Inhibition of the mitochondrial adenine nucleotide transport system by oleyl CoA. Arch Biochem Biophys 1972, 150:199–209
42. McLean P, Gumaa KA, Greenbaum AL: Long-chain Acyl CoAs, adenine nucleotide translocase and the coordination of the redox states of the cytosolic and mitochondrial compartments. FEBS Lett 1971, 17:345–350
43. Lerner E, Shug AL, Elson C, Shrago E: Reversible inhibition of adenine nucleotide translocation by long chain fatty acyl coenzyme A esters in liver mitochondria of diabetic and hibernating animals. J Biol Chem 1972, 247:1513–1519
44. Shug AL, Thomsen JH, Folts JD, Bittar N, Klein MI, Koke JR, Hutch PJ: Changes in tissue levels of carnitine and other metabolites during myocardial ischemia and anoxia. Arch Biochem Biophys 1978, 187:25–33
45. Whitmer JT, Idell-Wenger JA, Rovetto MJ, Neely JR: Control of fatty acid metabolism in ischemic and hypoxic hearts. J Biol Chem 1978, 253:4305–4309
46. Idell-Wenger JA, Grotyohann LW, Neely JR: Coenzyme A and carnitine distribution in normal and ischemic hearts. J Biol Chem 1978, 253:4310–4318
47. Hunter DR, Haworth RA, Southard JH: Relationship between configuration, function, and permeability in calcium-treated mitochondria. J Biol Chem 1976, 251: 5069–5077
48. Hunter DR, Haworth RA: The Ca^{2+}-induced membrane transition in mitochondria: 1. The protective mechanisms. Arch Biochem Biophys 1979, 195:453–459
49. Haworth RA, Hunter DR: The Ca^{2+}-induced membrane transition in mitochondria: II. Nature of the Ca^{2+} trigger site. Arch Biochem Biophys 1979, 195: 460–467
50. Hunter DR, Haworth RA: The Ca^{2+}-induced membrane transition in mitochondria: III. Transitional Ca^{2+} release. Arch Biochem Biophys 1979, 195:468–477
51. Pfeiffer DR, Schmid PC, Beatrice MC, Schmid HHO: Intramitochondrial phospholipase activity and the effects of Ca^{2+} plus N-ethylmaleimide on mitochondrial function. J Biol Chem 1979, 254:11485–11494
52. Beatrice MC, Palmer JW, Pfeiffer DR: The relationship between mitochondrial membrane permeability, membrane potential, and the retention of Ca^{2+} by mitochondria. J Biol Chem 1980, 255:8663–8671
53. Chien KR, Pfau RD, Farber JL: Ischemic myocardial cell injury: Prevention by chlorpromazine of an accelerated phospholipid degradation and associated membrane dysfunction. Am J Pathol 1979, 97:505–529
54. Farber JL, Martin JT, Chien KR: Irreversible ischemic cell injury: Prevention by chlorpromazine of the aggregation of the intramembranous particles of rat liver plasma membranes. Am J Pathol 1978, 92:713–732
55. Duppel W, Dahl G: Effect of phase transition on the distribution of membrane-associated particles in microsomes. Biochim Biophys Acta 1976, 426:408–417
56. Hochli M, Hackenbrock CR: Thermotropic lateral translational motion of intramembrane particles in the inner mitochondrial membrane and its inhibition by artificial peripheral proteins. J Cell Biol 1977, 72:278–291
57. James R, Branton D: Lipid- and temperature-depen-

dent structural changes in *Acholeplasma laidlawii* cell membranes. Biochim Biophys Acta 1973, 323:378-390
58. Kleeman W, McConnell HM: Lateral phase separations in *Escherichia coli* membranes. Biochim Biophys Acta 1974, 345:220-230
59. Rottem S, Yashouv J, Ne'eman Z, Razin S: Cholesterol in mycoplasma membranes: Composition, ultrastructure and biological properties of membranes from *Mycoplasma mycoides,* var *capii* cells adapted to grow with low cholesterol concentrations. Biochim Biophys Acta 1973, 323:495-508
60. Shechter E, Letellier L, Gulik-Krzywicki T: Relations between structure and function in cytoplasmic membrane vesicles isolated from an *Escherichia coli* fatty acid auxotroph: High angle x-ray diffraction, freeze-etch electron microscopy and transport studies. Eur J Biochem 1974, 49:61-76
61. Speth V, Wunderlich F: Membranes of *Tetrahymena:* II. Diector visualization of reversible transitions in biomembrane structure induced by temperature. Biochim Biophys Acta 1973, 291:621-628
62. Verkleij AJ, Ververgaert PHJ, Van Deenen LLM, Elbers PF: Phase transitions of phospholipid bilayers and membranes of *Acholeplasma laidlawii* B visualized by freeze fracturing electron microscopy. Biochim Biophys Acta 1972, 288:326-332
63. Wunderlich F, Wallach DFH, Speth V, Fischer H: Differential effects of temperature on the nuclear and plasma membranes of lymphoid cells: A study by freeze-etch electron microscopy. Biochim Biophys Acta 1974, 373:34-43
64. Thompson JE, Mittnacht S Jr, Farber JL: Unpublished observations
65. Papahadjopoulos D, Jacobson K, Nir S, Isac T: Phase transitions in phospholipid vesicles. Fluorescence polarization and permeability measurements concerning the effect of temperature and cholesterol. Biochim Biophys Acta 1973, 311:330-348
66. Blok MC, Van der Neut-Kok ECM, Van Deenen LLM, De Gier J: The effect of chain length and lipid phase transitions on the selective permeability properties of liposomes. Biochim Biophys Acta 1975, 406:187-196
67. Inoue K: Permeability properties of liposomes prepared from dipalmitoyllecithin, dimyristoyllecithin, eGG lecithin, rat liver lecithin and beef brain sphingomyelin. Biochim Biophys Acta 1974, 339:390-402
68. Fukuzawa K, Ikeno H, Tokumura A, Tsukatani H: Effect of alpha-tocopherol incorporation of glucose permeability and phase-transition of lecithin liposomes. Chem Phys Lipids 1979, 23:13-22
69. Adams RJ, Cohen DW, Gupte S, Johnson JD, Wallik ET, Wang T, Schwartz A: *In vitro* effects of palmitylcarnitine on cardiac plasma membrane Na,K-ATPase, and sarcoplasmic reticulum Ca^{2+}-ATPase and Ca^{2+} transport. J Biol Chem 1979, 254:12404-12410
70. Wildenthal K: Lysosomes and lysosomal enzymes in the heart. Lysosomes in Biology and Pathology. Vol 4. Edited by JT Dingle, RT Dean. Amsterdam, North Holland Publishing, 1975, pp 167-190
71. Hoffstein S, Gennaro DE, Weissmann G, Hirsch J, Streuli F, Fox AC: Cytochemical localization of lysosomal enzyme activity in normal and ischemic dog myocardium. Am J Pathol 1975, 79:193-206
72. Hoffstein S, Weissmann G, Fox AC: Lysosomes in myocardial infarction: Studies by means of cytochemistry and subcellular fractionation, with observations on the effects of methylprednisolone. Circulation 1976, 53(Suppl 1):I-34-I-40
73. Decker RS, Poole AR, Griffin EE, Dingle JT, Wildenthal K: Altered distribution of lysosomal cathepsin D in ischemic myocardium. J Clin Invest 1977, 59:911-921
74. Wildenthal K, Decker RS, Poole AR, Griffin EE, Dingle JT: Sequential lysosomal alterations during cardiac ischemia: I. Biochemical and immunohistochemical changes. Lab Invest 1978, 38:656-661
75. Decker RS, Wildenthal K: Sequential lysosomal alterations during cardiac ischemia: II. Ultrastructural and cytochemical changes. Lab Invest 1978, 38:662-673
76. Wildenthal K: Lysosomal alterations in ischemic myocardium: Result or cause of myocellular damage? J Mol Cell Cardiol 1978, 10:595-603
77. Ingwall JS, DeLuca M, Sybers HD, Wildenthal K: Fetal mouse hearts: A model for studying ischemia. Proc Natl Acad Sci USA 1975, 72:2809-2813
78. Decker RS, Wildenthal K: Lysosomal alterations in hypoxic and reoxygenated hearts: I. Ultrastructural and sysochemical changes. Am J Pathol 1980, 98: 425-444
79. Decker RS, Poole AR, Crie JS, Dingle JT, Wildenthal K: Lysosomal alterations in hypoxic and Reoxygenated hearts: II. Immunohistochemical and biochemical changes in cathepsin D. Am J Pathol 1980, 98:445-456
80. Weissman G, Zurier RB, Spieler PJ, Goldstein IM: Mechanisms of lysosomal enzyme release from leukocytes exposed to immune complexes and other particles. J Exp Med 1971, 134(Suppl):149S-165S
81. Allison AC, Harington JS, Birbeck M: An examination of the cytotoxic effects of silica on macrophages. J Exp Med 1966, 124:141-154
82. Nadler S, Goldfischer S: The intracellular release of lysosomal contents in macrophages that have ingested silica. J Histochem Cytochem 1970, 18:368-371
83. Sternlieb I, Goldfischer S: Heavy metals and lysosomes, Lysosomes in Biology and Pathology. Vol 5. Edited by JT Dingle, RT Dean. Amsterdam, North Holland Publishing 1976, pp 185-200
84. Allison AC, Young MR: Uptake of dyes and drugs by living cells in culture. Life Sci 1964, 3:1407-1414
85. Allison AC, Mallucci L: Histochemical studies of lysosomes and lysosomal enzymes in virus-infected cell cultures. J Exp Med 1965, 121:463-476
86. Kane AB, Stanton RP, Raymond EG, Dobson ME, Knafelc ME, Farber JL: Dissociation of intracellular lysosomal rupture from the cell death due to silica J Cell Biol 1980, 87:643-651
87. Van der Bosch H: Phosphoglyceride metabolism. Ann Rev Biochem 1974, 43:243-277
88. Waite M, Sisson P: Partial purefication and characterization of the phospholipase A_2 from rat liver mitochondria. Biochemistry 1971, 10:2377-2383
89. Scherphof GL, Waite M, Van Deenen LLM: Formation of lysophosphatidyl ethanolamines in cell fractions of rat liver. Biochim Biophys Acta 1966, 125: 406-409
90. Nachbaur J, Colbeau A, Vignais PM: Distribution of membrane-confined phospholipases A in the rat hepatocyte. Biochim Biophys Acta 1972, 274:426-446
91. Newkirk JD, Waite M: Identification of a phospholipase A_1 in plasma membranes of rat liver. Biochim Biophys Acta 1971, 225:224-233
92. Weglicki WB, Waite M, Sisson P, Shohet SB: Myocardial phospholipase A of microsomal and mitochondrial fractions. Biochim Biophys Acta 1971, 231:512-519
93. Bjørnstad P: Phospholipase Activity in rat liver microsomes studied by the use of endogenous substrates. Biochim Biophys Acta 1966, 116:500-510
94. Sherman SC, Chien KR, Mittnacht S Jr, Farber JL: Microsomal membrane structures and function subsequent to calcium activation of an endogenous phospholipase. Arch Biochem Biophys 1980, 205:614-622
95. Nayler WG, Poole-Wilson PA, Williams A: Hypoxia and calcium. J Mol Cell Cardiol 1979, 11:683-706