Cardiol

POST-ISCHEMIC MYOCARDIAL FUNCTION

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INTRODUCTION

In 1935 Tennant and Wiggers (Tennant and Wiggers, 1935) very elegantly and carefully described the mechanical responses of the left ventricle to the interruption of coronary artery blood flow. Conclusion number three described the development of regional ischemic dyskinesis, that is, the replacement of normal systolic myocardial fiber shortening and wall thickening by systolic myocardial fiber lengthening with consequent wall thinning in a myocardial region deprived of its blood supply.

"Occlusion of a main coronary branch is followed by an evoluting series of myographic changes which indicate progressive enfeeblement of contraction to the extent that approximately within a minute the area stretches during isometric contraction, remains stretched during systolic ejection and shortens quickly during isometric relaxation; in short, the myogram is completely inverted. Similar changes in contraction of the right ventricle occur following ligation of the right coronary artery."

While conclusion number three is often cited as the most important observation made in this study, in fact, the authors made another equally important observation which has been largely overlooked. This observation, conclusion number four, described the functional consequences of <u>restoration</u> of blood flow to a myocardial region made dyskinetic by ischemia.

"(Following coronary occlusion) reestablishment of the normal blood supply is followed by a reversed series of myographic changes with restoration of normal contractions provided the period of ischemia is not too long in duration."

Since the publication of this paper, the relationship between transient regional myocardial ischemia and contractile function has been shown to be considerably more complex than this conclusion would imply. However, the general principle that <u>timely</u> restoration of blood flow results in anatomic and functional salvage of ischemic myocardium remains true (Theroux, 1976; Reimer, 1979; Lavallee, 1983). Identification of the role of coronary artery thrombus formation in the genesis of acute myocardial infarction and the current feasibility of clinical thrombolysis by streptokinase, urokinase, tissue plasminogen activator or percutaneous transluminal coronary angioplasty have made the properties of myocardium "salvaged" by reperfusion a critical clinical matter rather than a laboratory observation. What has become obvious is that functional and biochemical recovery of viable myocardium after ischemia are significantly delayed, that reperfusion itself may have separate deleterious effects which may be superimposed upon ischemic injury and finally, that ischemic duration is not the sole variable influencing post-ischemic myocardial function.

Thus, despite the complexity of the relationship between restoration of coronary artery blood flow and myocardial contractile function, understanding the determinants of post ischemic myocardial function and thereby altering therapy to enhance the contractile performance of "reperfusion-salvaged" myocardium has become an important clinical cardiologic challenge.

CLINICAL RESULTS OF THROMBOLYSIS

While many clinical studies demonstrate the feasibility of early thrombolysis during acute myocardial infarction (Andersen et al., 1983; Kennedy et al., 1983; Khaja et al., 1983; Anderson et al., 1984; Collen et al., 1984; Leiboff et al., 1984; Rentrop et al., 1984; Van de Werf et al., 1984; Holmes et al., 1985; Relman, 1985; Rentrop, 1985; Topol et al., 1985; Erbel et al., 1986; Fung et al., 1986; Gissi Study Group, 1986; ISAM Study Group, 1986; Jang et al., 1986; O'Neill et al., 1986; Serruys et al., 1986; Simoons et al., 1986), the fate of the myocardium distal to the recanalized vessel remains controversial. Improvement in global or regional left ventricular function and decreases in mortality following reperfusion have not been unequivocally demonstrated.

The following chart summarizes the results of several selected trials of thrombolysis during acute myocardial infarction. These trials were selected because they are randomized, controlled trials and clearly demonstrate the heterogeneity of outcome following clinical thrombolysis with respect both to left ventricular function and to patient mortality. What is obvious from this, is that despite similar ischemic durations, significant improvement in ejection fraction and mortality are not uniform.

Streptokinase-Randomized Trials

Intracoronary Streptokinase

Study	ICSK(n)	Control(n)	% Recanal.	Time to Infusion(n)	EF % ICSK	EF % Control	P	Mortality % ICSK	Mortality % Control	P
Anderson (1983)	24	26	75	4.0±0.75	+3.9±4.6	-3.0±8.4	<0.001	4	15	NS
Khaja (1983)	20	20	60	5.4±1.5	+3.0	+2.0	NS	5	10	NS
Kennedy (1983)	134	116	68	4.6±2.2	1	0	NS	3.7	11.2	<0.02
Leikoff (1983)	22	18	69	4.0	-2.8%	-0.4%	NS	9	5	NS
Rentrop (1984)	23	24	74	5.9	+2.1%	-1.4	NS	21	10	NS
Serruys (1986)	269	264	79	3.3	53%	47%	<0.0001	----	----	----
Simoons (1986)	269	264	79	3.3	----	----	----	5	9	<0.05

Intravenous Streptokinase

Italian Gissi (1986)	5,860	5,853	----	<12 hours	----	----	----	10.7	13	<0.0002
German-Swiss Canadian ISAM	859	882	----	<6 hours	56.8	53.9	<0.005	6.3	7.1	NS

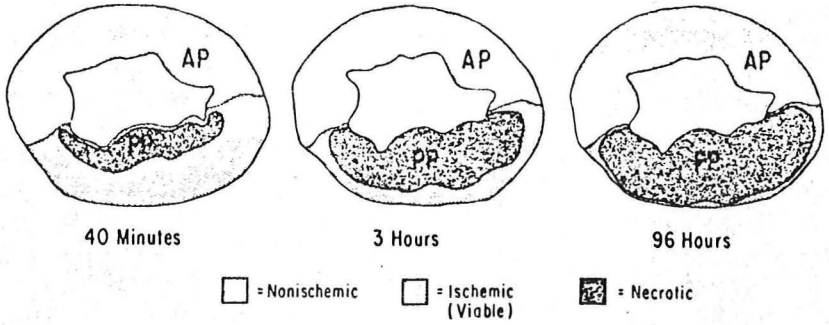
A more recent approach to restoration of blood flow has combined thrombolysis with mechanical recanalization of the occluded vessel by percutaneous coronary angioplasty (Erkel, 1986; Fung, 1986; O'Neill, 1986). The rationale for this approach is twofold: 1. to provide more normal blood flow by further decreasing residual stenosis following clot dissolution and 2. to decrease the incidence of coronary artery reocclusion. Myocardial consequences of this combined approach are still to be defined, however early reports of small numbers of patients are encouraging.

Despite the variable results of these clinical trials, there is very compelling laboratory evidence to support both anatomic and functional myocardial salvage by restoration of coronary blood flow.

ANATOMIC AND FUNCTIONAL CONSEQUENCES OF PERMANENT CORONARY ARTERY OCCLUSION

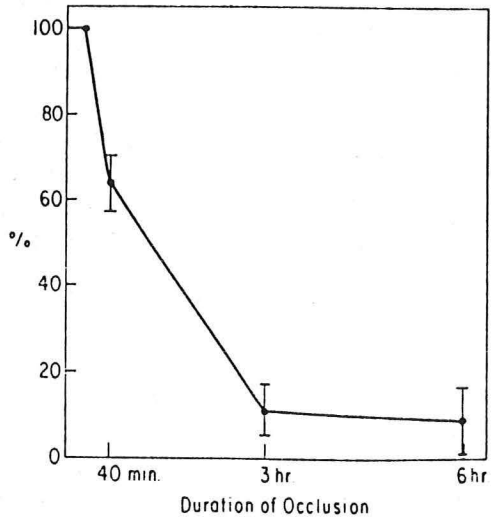
A. Infarct Size Following Permanent Coronary Artery Occlusion

In animal studies following permanent occlusion of a coronary artery, an area of myocardial ischemia, the "area-at-risk" or "risk region," is created. Within this area, myocardial blood flow is reduced but in a non-uniform fashion - there is relatively more reduction in the subendocardial flow ($3\pm0.5\%$ control flow) than in the subepicardial flow ($17\pm1.6\%$ control flow) (Reimer, 1979; Marcus, 1983). Myocardial cell death in the area-at-risk therefore takes place in a non-homogeneous fashion, closely coupled to the degree of blood deprivation. Thus, cell death proceeds sequentially from the relatively more flow deprived subendocardium to the subepicardium (Reimer, 1977; Reimer, 1979), the so-called "wavefront" of cell death (Reimer, 1979). However if severe ischemia is sustained for a sufficient length of time, transmural necrosis will occur (Reimer, 1977). The period of time required for transmural necrosis varies with such factors as species, collateral blood supply and myocardial oxygen consumption.



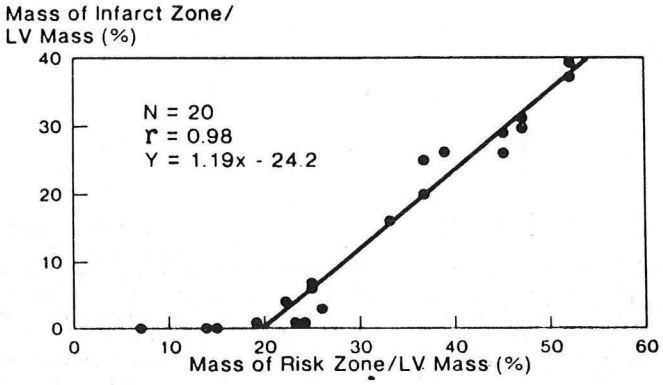
Reimer and Jennings, 1979.

Final infarct size is directly proportional both to the size of the region at risk (Marcus, 1983) and to the duration of ischemia.



. Salvageable ischemic myocardium; myocardium salvaged by reperfusion (as a percentage of the ischemic LCC bed) is plotted with respect to the duration of ischemia. Permanent occlusion caused necrosis involving 79 per cent of the LCC bed. Thus, 79 per cent of the LCC bed was considered to be at risk in the reperfusion studies. Reperfusion at 40 minutes resulted in necrosis averaging 28 per cent of the LCC bed and 28 of 79 = 36 per cent of the permanent infarct size. Thus, reperfusion at this time salvaged 64 per cent of the myocardium at risk. By this group analysis, little or no salvage was achieved by reperfusion after 3 or 6 hours of ischemia.

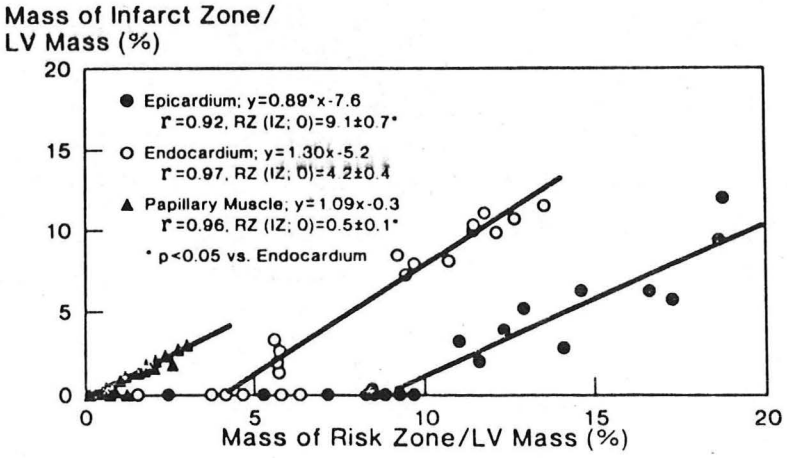
Reimer and Jennings, 1979.



. Relationship between mass of infarct area and mass of risk area for whole heart. Each mass is normalized by total left ventricular (LV) mass. Note significant relationship between the 2 measurements. Horizontal axis intercept indicates that infarction does not occur when risk region is less than about 20% of left ventricle.

Koyanagi et al., 1982.

The relationship between area at risk and infarction has been further broken down by myocardial layers from endocardium to epicardium.



Relationship between mass of myocardial infarction zone/left ventricular (LV) mass and mass of risk zone/LV mass at subepicardium, subendocardium, and papillary muscle. Regression equation, correlation coefficient, and intercept on horizontal axis (mass of risk zone where infarct mass is 0) are shown for each region. Note that slope of relationship is steeper for subendocardium than subepicardium, and intercepts on horizontal axis are significantly different among the 3 regions.

Koyanagi et al., 1982.

While the slopes and intercepts of these lines are different, the relationship in any given myocardial wall layer remains a linear one.

B. Ultrastructural Changes During Permanent Ischemia

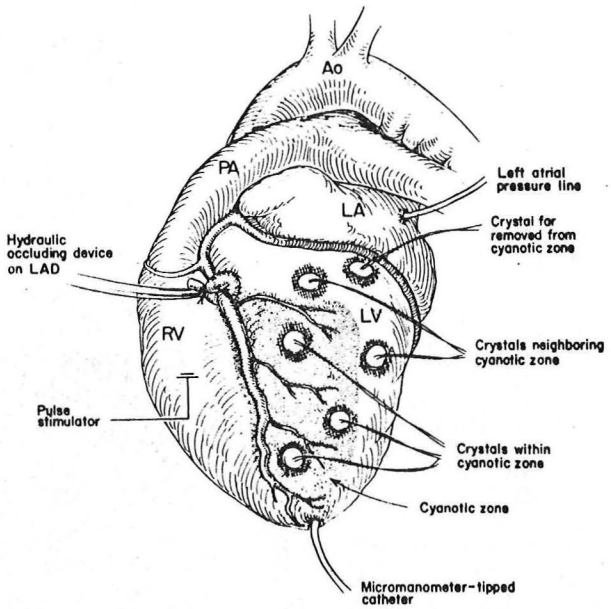
During the development of myocardial infarction, cells in the central ischemic zone undergo a series of alterations which progress to irreversible injury. These have been well characterized by numerous investigators (Jennings et al., 1965, Jennings and Ganote, 1974, Jennings et al., 1975 and Schaper and Schaper, 1986). Thus, early after ischemia, there is glycogen depletion margination of nuclear chromatin, mitochondrial swelling, and depletion of high energy phosphates (Reimer et al., 1981) findings which resolve if blood flow is restored (Basuk, 1986). As ischemia is sustained, structural derangements become more severe, sarcolemmal defects become apparent, cell volume and ion regulation are lost followed by calcium overload and membrane degradation. At this point, the changes are no longer reversible by reperfusion. Thus, prior to irreversible cell injury, there is a window of time during which restoration of blood flow results in anatomic salvage of myocardial cells.

C. Regional Function Following Permanent Coronary Artery Occlusion

Normal myocardial function is marked by myocardial fiber shortening during systole with consequent wall thickening. The degree of shortening and therefore wall thickening normally varies regionally both from apex to base and around the circumference of the left ventricle. Additionally, one can demonstrate temporal heterogeneity of contraction so that not all regions of the heart reach end-systole simultaneously (Gallagher, 1985; Taylor and Kerber, 1985). Disturbances in regional function in an area of myocardial ischemia depend on the transmural extent and severity of ischemia, with dyskinesis (paradoxic systolic wall bulging) or akinesis (absence of normal wall

thickening) predicting transmural severe reductions in blood flow while hypokinesis (depressed systolic wall thickening) is associated with either non transmural ischemia or normal myocardium (Gallagher, 1980; Weiss, 1981).

When one examines regional function closely following permanent coronary artery occlusion, there is a close interrelationship among decreases in regional blood flow and contractile function (Theroux, 1976; Roan, 1979; Gallagher, 1980; Roan, 1981). In studies using a canine model, pairs of ultrasonic dimension gauges were implanted in the left ventricular myocardium in an endocardial to epicardial orientation to measure regional myocardial systolic thickening or in a side by side orientation to measure myocardial systolic fiber shortening. Regional myocardial blood flow was measured by radioactive microspheres.



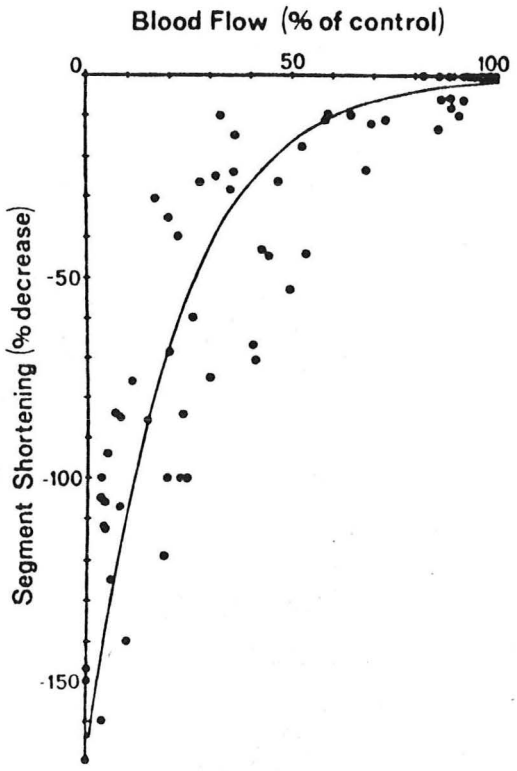
LAD occlusion is produced by inflation of a hydraulic occluding device encircling the LAD and producing an area of ischemia. Ultrasonic pulse-transit piezoelectric crystals are inserted to measure wall thickness within, near, and well removed from the cyanotic area.

Roan et al., 1979.

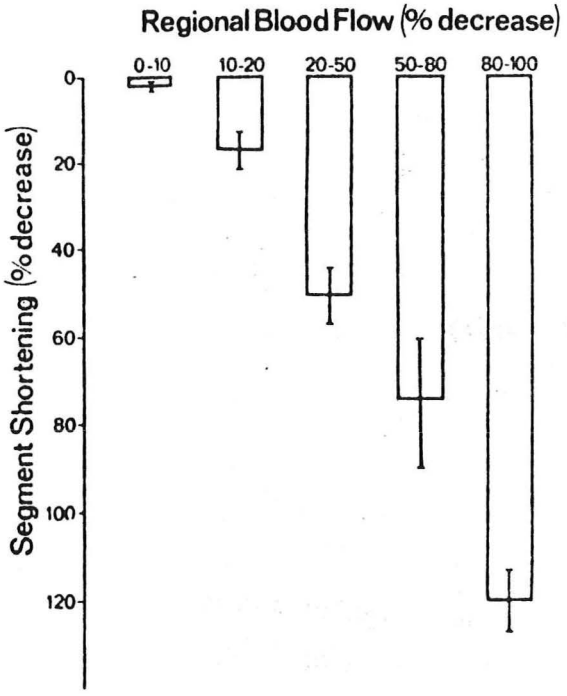
In general, as regional myocardial blood flow decreases, wall motion becomes increasingly abnormal, although not in a perfectly linear fashion (Gallagher, 1980; Vatner, 1980). Thus, Gallagher (1980) found that if significant decreases in perfusion to the inner one half of the ventricular wall occurred, a 75% reduction in systolic thickening occurred. If significant decreases in flow occurred over three quarters of the transmural extent of the ventricular wall (i.e. if flow was normal in only the outer one fourth of the ventricular wall) no thickening (akinesis) occurred. Dyskinesis or aneurysmal bulging of the ventricular wall occurred when flow was severely decreased

across the entire ventricular wall. Thus function is closely, though not perfectly linearly, related to coronary blood flow, with the contribution of the subendocardium having greater importance.

Vatner (1980) demonstrated that the relationship between decreases in blood flow and decreases in regional function was best described by an exponential function.



The exponential relationship between % decreases in regional segment length (SL) shortening (ordinate) are plotted against decreases in regional myocardial blood flow (BF) as % control (abscissa): SL(%Δ) = −161.6 e$^{-0.04\,BF(\%)}$ (r = 0.92).

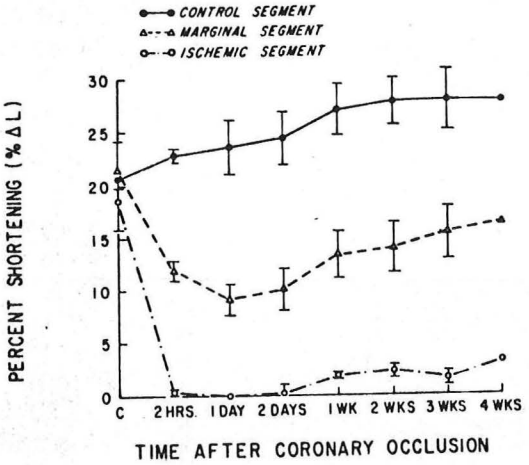


Segments grouped according to % decreases in regional blood flow (abscissa) show progressive decreases in regional myocardial function (ordinate). Segments with 10-20% decreases in regional blood flow show significant decreases in function.

Vatner, 1980.

Of interest in this study, segment length rather than thickening was measured by placing pairs of dimension gauges side by side rather than in the endocardial to epicardial orientation. In this fashion the relationship between flow and function was examined in regions of homogeneous flow (subendocardium only) rather than across a region of heterogeneous flow (subepicardium to subendocardium).

When the evolution of regional function over time following permanent occlusion is examined, the following becomes apparent. Using similar techniques, regional responses over a 1 week period were characterized by Roan et al. (1979). Myocardial segments were subdivided into three classes depending on function as related to pre-ischemic function: those with 67-100% of pre-ischemic systolic thickening at 5 minutes ischemia, those with 0-67% of systolic thickening (hypokinetic segments) and those with less than 0% control function (dyskinetic segments). Between 5 minutes and 24 hours of ischemia, there was virtually no change in function in segments of each of the three classes. Between 24 hours and one week after permanent coronary artery occlusion, there was very little further evolution in function in these classes of segments. The only change reported was a decrease in the degree of aneurysmal bulging, but not replacement of dyskinesis by systolic thickening of any degree. The decrement in aneurysmal bulging may reflect some functional recovery but could be accounted for by alterations in the elastic properties of the evolving scar. Theroux (1977) extended the period of observation of regional function following permanent coronary artery occlusion up to four weeks using similar techniques but measuring myocardial fiber length change rather than wall thickening. When fiber length is used as a gauge of normal function, normal change is systolic fiber shortening and ischemic dysfunction is manifested by systolic fiber lengthening. Over a four week period, these authors also found very little improvement in segmental shortening.



Percent changes in end-diastolic segment dimensions (upper panel) and in the percentage shortening of the three segments (lower panel) at various intervals after permanent circumflex coronary artery occlusion in unanesthetized dogs.

Theroux et al., 1977.

The major points of importance in these experiments are: 1) that following permanent coronary artery occlusion, the degree of dysfunction is related to the intensity of the ischemia, and, 2) that the degree of dysfunction early after permanent coronary artery occlusion is predictive of subsequent function. Because there is very little change in wall motion following permanent coronary artery occlusion, early assessment of the extent of dyskinesis (aneurysmal systolic bulging) can be used to estimate the size of transmural myocardial infarction (Weiss, 1981; Wyatt et al., 1981; Pandian, 1983).

When amount of segmental necrosis was compared to segmental function in this model of permanent coronary artery occlusion, a correlation between abnormalities of segmental function and extent of necrosis (Roan, 1981) was demonstrated.

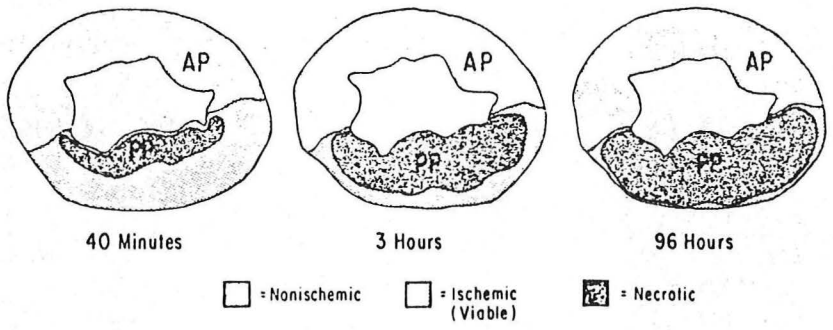
Endocardial necrosis was always more severe than midwall and epicardial necrosis paralleling the distribution of regional blood flow in the ischemic zone. This was true whether a segment was hypokinetic or dyskinetic.

While this relationship is linear, it should be noted that one need not have one hundred percent necrosis to have severe dysfunction.

ANATOMIC AND FUNCTIONAL CONSEQUENCES OF TRANSIENT ISCHEMIA

A. Infarct Size in Reperfused Myocardium

There is ample evidence that timely reperfusion significantly reduces infarct size in animal studies (Ginks, 1972; Maroko, 1972; Costantini, 1975; Baughman, 1981; Geary, 1982; Heyndrickx, 1985). This was true whether dogs, baboons or pigs were studied. The importance of time in myocardial salvage was best shown by Reimer and Jennings (1979). Using a canine model, these investigators performed a circumflex coronary artery ligation and then allowed for reperfusion at 40 minutes, 3 hours, and 6 hours of coronary artery occlusion. A group of animals in whom permanent coronary artery occlusion was performed served as controls. At four days post occlusion, all dogs were sacrificed and infarct size was measured as percent of the region at risk. After 40 minutes of ischemia 28 percent of the risk region was infarcted, at 3 hours 70 percent, at 6 hours 72 percent and when no reflow occurred 79 percent of the risk region was infarcted. Necrosis proceeded from endocardium to epicardium in all instances.



Reimer and Jennings, 1979.

Most other canine studies support the concept that three hours is the window of time within which significant myocardial salvage occurs with reperfusion in the canine model (Ginks, 1972; Baughman, 1981; Lavallee, 1983).

B. Ultrastructural Changes With Transient Ischemia

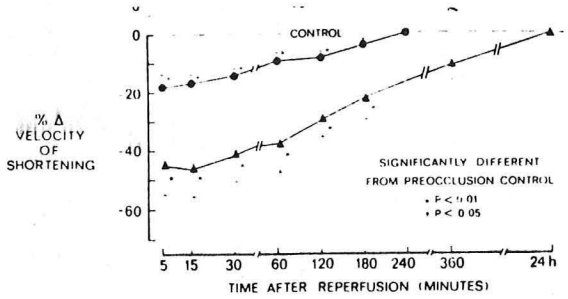
With transient myocardial ischemia, distinctive ultrastructural changes are observed, all of which become increasingly severe with increasing ischemic duration (Jennings et al., 1965; Jennings and Gonate, 1974; Schaper et al., 1979). Prominent changes include early glycogen depletion, margination of nuclear chromatin and mitochondrial alterations. With time, sarcolemmal damage becomes apparent (Schaper et al., 1979; Schaper and Schaper, 1983;). Cell architectural changes resulting from as little as 10 minutes of ischemia were completely reversed by 20 minutes of reperfusion (Basuk, 1986; Reimer and Jennings, 1986). When four separate 10 minute occlusions were performed with 20 minutes of reperfusion between each occlusion, cell architecture was restored to normal except for the presence of rare swollen mitochondria. Thus forty minutes of ischemia separated by periods of reperfusion did not result in injury. Of interest is that cell repair following transient ischemia also follows a transmural gradient paralleling transmural distribution of blood flow. When blood flow is restored, subendocardial blood flow is depressed compared to subepicardial flow. Histologic repair lags in the subendocardium (Schaper et al., 1979) paralleling blood flow distribution. When ischemia lasted 40 minutes or more without periods of reflow, features of irreversible injury were present. Such irreversibly injured cells sustained further structural damage consisting of marked calcium accumulation, loss of volume regulation with severe edema, and membrane degradation (Chien et al., 1984) upon reperfusion. When irreversibly injured cells are exposed to normotonic reperfusate they undergo explosive swelling and severe disruption of cellular elements (Schaper et al., 1979).

While the features of irreversible injury are well characterized, the precise events which precipitate the change from a reversibly to an irreversibly injured state are as yet unclear.

C. Functional Effects of Reperfusion

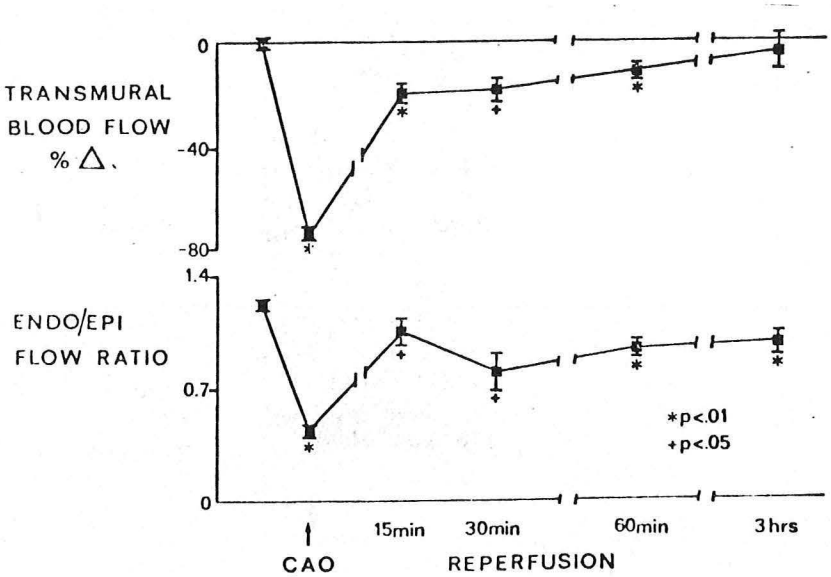
The contractile function of ischemic myocardium is also benefited by restoration of blood flow. Heyndrickx (1975) and colleagues described the regional myocardial contractile responses to brief periods of coronary artery occlusion. These investigators used chronically implanted dimension gauges to measure regional myocardial fiber length changes, velocity of shortening, and blood flow velocity in a selected coronary artery during brief occlusion periods of 5 and 15 minutes. Additionally, they monitored ST segment changes. They found that with coronary occlusion, there was pronounced ST segment elevation in the ischemic zone, depression of coronary blood flow, and severe depression of regional function. With release of occlusion and reperfusion, there was prompt resolution of ECG changes and normalization of coronary blood flow velocity in the artery which had been occluded. However, prolonged

depression of muscle function as assessed by measurement of velocity of segmental shortening (Vcf) was noted.



Heyndrickx et al., 1975.

As can be seen from the above diagram, regional function remained depressed for several hours. After five minutes of ischemia, three hours were required for a return to control Vcf, while after fifteen minutes of ischemia, up to 24 hours were required for return to control. Thus, despite the fact that ischemia was resolved, that the ECG was normalized, there remained significant and prolonged functional derangement. In an effort to understand the mechanism of prolonged dysfunction, these same investigators examined the distribution of coronary blood flow in the ischemic zone during coronary artery occlusion of 15 minutes and up to 24 hours of reperfusion. Their functional and blood flow findings are summarized on the diagram below.



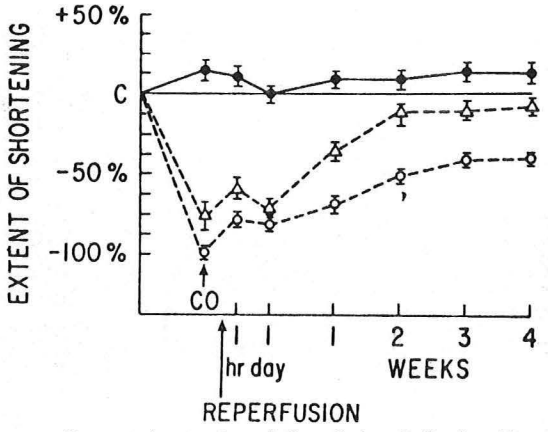
Effects of a 15-min coronary occlusion and subsequent reperfusion on average (± SEM) changes of transmural myocardial blood flow (*top panel*) and the endo/epi flow ratio for all ischemic-zone segments studied. Significant changes are denoted by symbols. After reperfusion, when reactive hyperemia had subsided, transmural flow remained depressed for 1 h and the endo/epi ratio remained depressed for at least 3 h.

Heyndrickx et al., 1978.

Prolonged functional depression in the absence of evidence of histologic necrosis was demonstrated; in addition they found depression in regional myocardial blood flow and in the ratio of blood flow to the endocardium and epicardium. Thus, transmural distribution of blood was abnormal up to 3 hours after a 15 minute ischemic period. While this seemed a reasonable explanation for functional abnormalities, an alternative explanation for the finding may be that the reduction in blood flow was in response to the reduction in mechanical function since myocardial blood flow is closely autoregulated to match workload.

The studies of Heyndrickx showed clearly that the duration of functional impairment depended upon the duration of ischemia when reperfusion followed brief coronary occlusion periods. While these studies have implications regarding the effects of episodes of angina in patients, they did not shed light on potential consequences of reperfusion after a longer period of ischemia such as would be seen if thrombolysis interrupted an acute myocardial infarction.

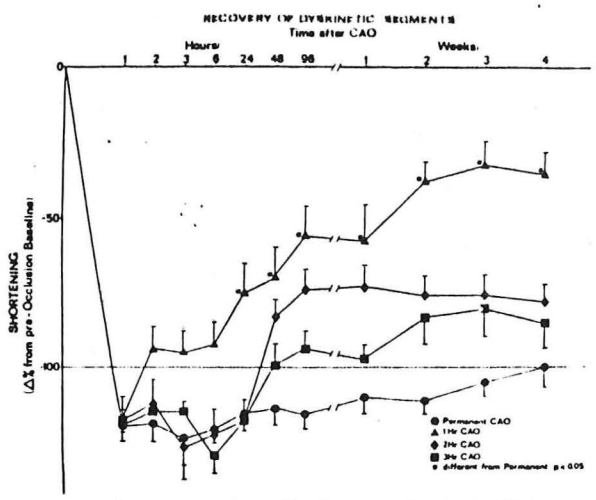
Theroux et al. (1976) examined the regional responses using similar techniques following two hours of coronary artery occlusion. Their findings are summarized by the diagram below.



Percent changes in end-diastolic length (top) and in extent of shortening (bottom) for the five dogs studied. The changes from control (C) are shown for the occlusion period (CO) (2 hours) and the follow-up period after reperfusion (1 hour, 1 day and 1 to 4 weeks). Mean values ± standard error of the mean are shown.

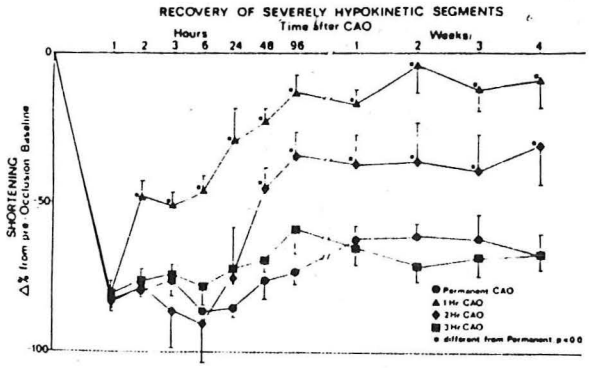
Theroux et al., 1976.

Similarly, Lavallee et al. (1983) compared systolic contractile recovery over a four week period in four groups of dogs - dogs with 1, 2, or 3 hours of coronary artery occlusion and a control group with permanent occlusion. His findings are summarized by the two diagrams below.

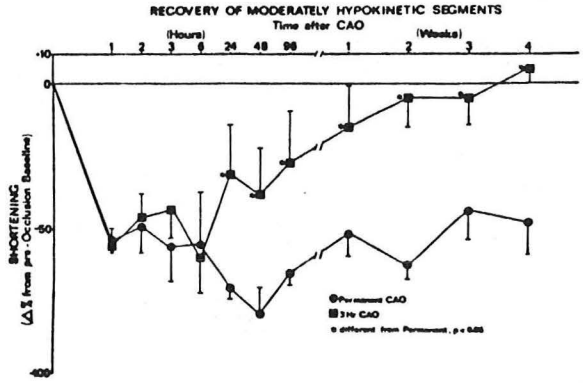


Time course of systolic shortening in the dyskinetic segments in the P group (circles) or 1-hour group (triangles), 2-hour group (diamonds), and 3-hour group (squares). Systolic shortening is expressed as percent change from pre-coronary artery occlusion (CAO) baseline. Values depressed by over 100% represent paradoxical systolic motion. The asterisks represent responses different from those in the P group.

Lavallee et al., 1983.



Time course of systolic shortening in the severely hypokinetic segments in P group (circles), or 1-hour group (triangles), 2-hour group (diamonds), and 3-hour group (squares). Systolic shortening is expressed as percent change from pre-coronary artery occlusion (CAO) baseline. The asterisks represent responses different from those in the permanent CAO group.



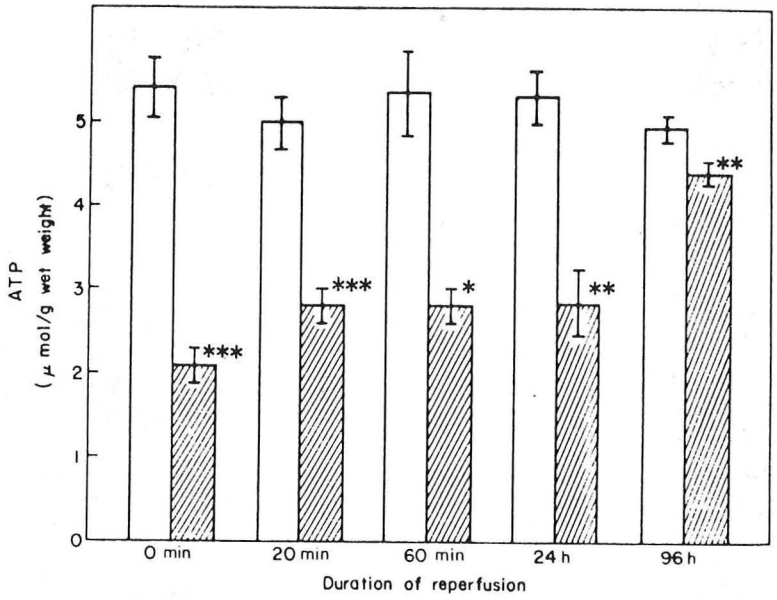
7. *Time course of systolic shortening in the moderately hypokinetic segments in the 3-hour (squares) and P (circles) groups. Systolic shortening is expressed as percent change from pre-coronary artery occlusion (CAO) baseline. The asterisks represent responses different from the permanent CAO group.*

Lavallee et al., 1983.

As was true with brief periods of ischemia, the extent and time course of recovery were closely coupled to the duration of the preceding ischemic period as well as to the severity of the ischemic dysfunction. Thus, after several hours of ischemia, weeks were required before functional recovery was complete. In contrast to the findings after permanent coronary artery occlusion, early ischemic function did not necessarily predict post reperfusion function. After transient ischemia, the extent of wall motion abnormality early after reperfusion does not predict final wall motion abnormality (Taylor et al., 1985), making estimates of infarct size based on this index more difficult.

Effects of Ischemia on Energy Stores

Because contraction is an energy requiring process, it is important to assess the effects of ischemia on energy stores. Neill et al. (1986) showed persistent abnormalities of ATP metabolism with sustained moderate ischemia (flow equaling 20 to 70% of control coronary flow). Thus complete coronary artery occlusion was not necessary to alter ATP and adenine nucleotides stores. High energy phosphate stores are rapidly depleted during brief periods of total ischemia. Thus, Reimer et al. (Reimer et al., 1981) examined the fate of ATP and the adenine nucleotide pool during a coronary artery occlusion of 15 minutes followed by reperfusion periods of 20 or 60 minutes, and 24 or 96 hours. The effect on ATP and phosphocreatine content are shown on the diagram below.

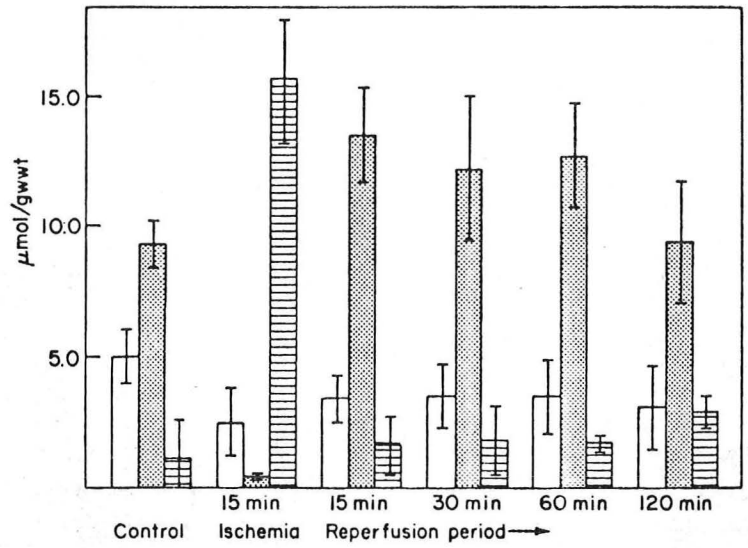


The effects of 15 min of ischemia with 0, 20, or 60 min or 24 or 96 h of reperfusion on ATP content is shown. ATP was markedly reduced in ischemic samples at all five times studied. ATP content increased slightly during the first 15 min of reperfusion most likely because of re-phosphorylation of ADP and AMP. ATP content showed no additional increase over the next 24 h and was still slightly decreased at 96 h. Brackets indicate ± S.E.M. Nonischemic and ischemic samples from each dog were compared by paired t statistical analysis. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Comparisons between groups using a non-paired t test showed significant increases in ATP with all times of reperfusion compared to no reperfusion ($P < 0.05$). ▨, Ischemic; □, Nonischemic.

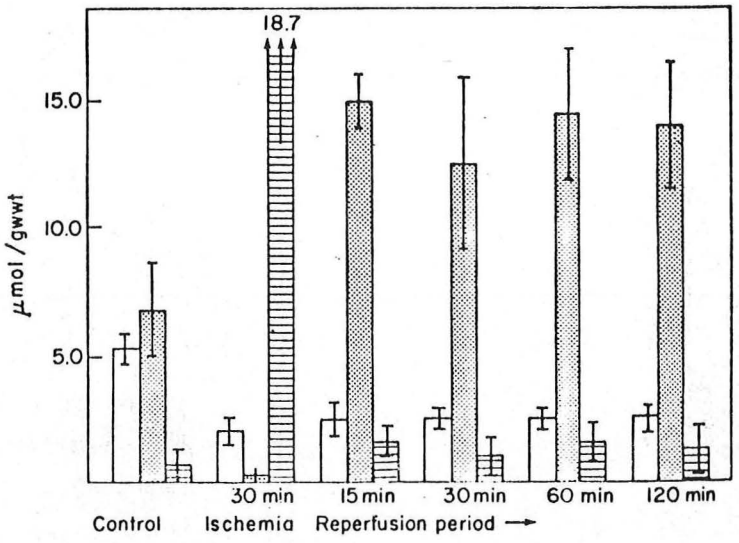
Reimer et al., 1981.

As can be seen, at 15 minutes of ischemia, there was a 60% depletion of ATP. ATP recovered rapidly to about 50% of control by 20 minutes of reperfusion and then remained depressed at this level for the ensuing 24 hours. By 96 hours of reperfusion, recovery was 91% of control, still slightly, but significantly depressed. A similar effect was seen when total adenine nucleotide pool was examined.

Studies using longer occlusive periods found both greater depression of ATP and less repletion (Schaper et al., 1979). At the point of irreversible injury, ATP and phosphocreatine levels are extremely low and there is little phosphocreatine rebound with reperfusion.

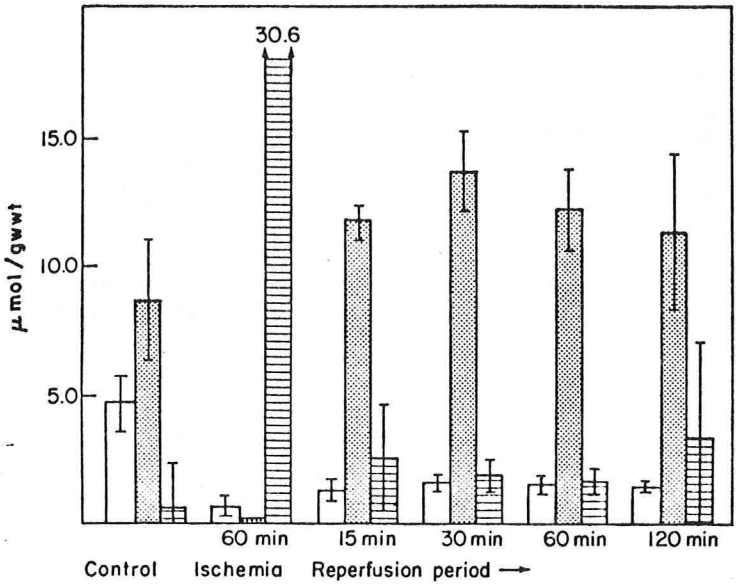


Content of ATP (□), phosphocreatine (▦) and lactate (▤) after slight (15 min) ischemia and during reperfusion.

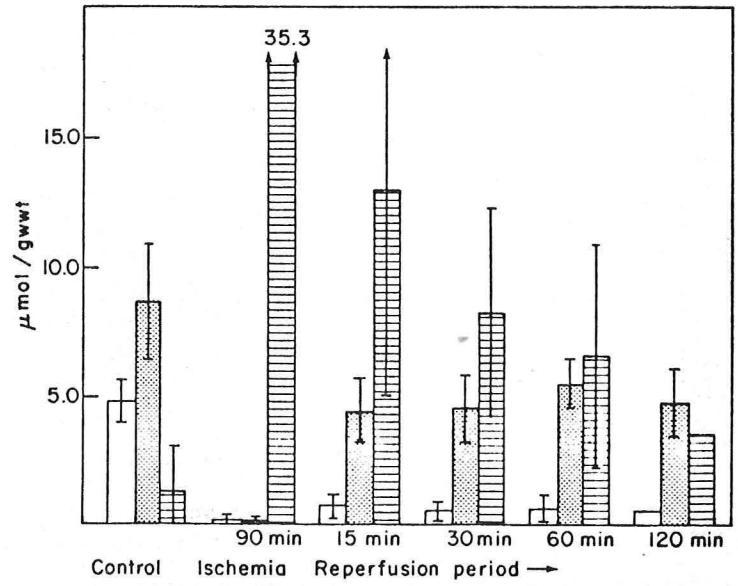


Content of ATP (□), phosphocreatine (▦) and lactate (▤) after moderate (30 min) ischemia and during reperfusion.

Schaper et al., 1979.



. Content of ATP (□), phosphocreatine (▥) and lactate (▤) after severe (60 min) ischemia and during reperfusion.



. Content of ATP (□), phosphocreatine (▥) and lactate (▤) after irreversible (90 min) ischemia and during reperfusion.

Schaper et al., 1979.

Thus, high energy phosphate depletion and slow repletion occurs with ischemia and reperfusion. While the depletion of ATP is related to the previous ischemic period, the slow repletion may be in part an effect of reperfusion, i.e. wash out of substrate (particularly adenosine, inosine and hypoxanthine) for ATP formation by the so called "salvage pathways" (Mauser et al., 1985). Enhanced utilization of ATP during cell repair processes may add to the prolonged depression of ATP levels (Mauser, 1985; Swain, 1985). If reperfusion-induced wash out of substrate in part accounts for prolonged depletion of ATP, then reperfusion with substrate to enhance ATP production is a logical experiment. This has been done in canine models and has resulted in accelerated resynthesis of high energy phosphate (Swain et al., 1982; Mauser et al. 1985). The relationship between enhanced repletion of ATP and recovery of function remains to be defined.

It has been proposed that when sufficient ATP depletion occurs, membrane integrity cannot be maintained and cell viability is lost (Chien et al., 1984). Thus, ATP depletion may be the crucial event in precipitating irreversible injury.

The explanation for the delay in functional and metabolic recovery has been sought by two lines of inquiry - first, that metabolic and structural consequences of the previous period of ischemia are repaired slowly paralleling contractile recovery; secondly, that reperfusion itself may have added a component of injury to ischemic, but potentially viable cells, thus prolonging recovery of function.

Deleterious Effects of Reperfusion

While reperfusion is the sine qua non for tissue survival, it provides the substrate for a new set of events which may themselves superimpose a component of injury. The tissue which is at risk for such injury is that tissue with advanced but still reversible injury. Thus there remains a question of whether the area of tissue necrosis is actually enlarged by the process required for tissue salvage. One potential adverse consequence of reperfusion has already been mentioned - that is, the wash out of substrate for high energy phosphate generation. Myocardial cell calcium accumulation is a second such effect.

When ischemic myocardium is reperfused, calcium accumulates in the cell and particularly in mitochrondria (Shine et al., 1978; Tranum-Jensen et al., 1980; Bourdillon and Poole-Wilson, 1981; Shine and Douglas, 1982; Ashraf et al., 1984; Cheung, 1986). Potential sources for this calcium are increased

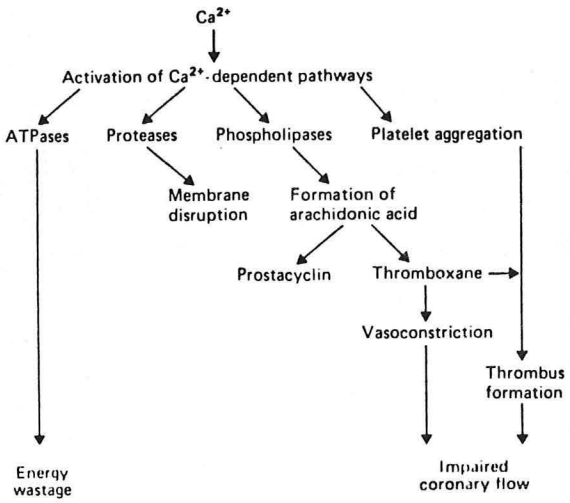
membrane channels or via membrane defects secondary to ischemic membrane injury. Decreased efflux from the cell due to damage to the Na^{+}-Ca^{++} exchange system or inappropriate intracellular Ca^{++} compartmentalization (Hammond and Hess, 1985) might contribute to calcium overload. While calcium is very importantly involved in such cell processes as excitation contraction coupling and regulation of intracellular enzymes systems, increased intracellular Ca^{++} has been associated with activation of Ca^{++} dependent phospholipases resulting in membrane degradation (Chien, 1978), increased Ca^{++}-ATPase activity resulting in loss of ATP above that lost during the ischemic process and uncoupling of mitochondrial oxidative phosphorylation. Additionally, calcium may potentiate the mitochondrial injury attributed to oxygen free radicals generated during the reoxygenation of ischemic tissue (Cheung, 1986).

The proof that calcium loading during reperfusion of ischemic myocardium is deleterious to mechanical function as well as to biochemical recovery has best been shown in experiments in which reperfusion is accomplished with solutions containing reduced calcium content. Shine et al. (1978) used isolated rabbit interventricular septae made ischemic for 60 minutes then reperfused with solutions containing either 1.5 mM CaCl or 0.75 mM $CaCl_2$. The difference in recovery was striking. The septae reperfused with low Ca^{++} content recovered 67.1 $\pm$ 7.5% control developed tension versus recovery of 31.6 $\pm$ 9.1% control developed tension in those septae perfused with 1.5 mM CaCl. Resting tension, a measure of myocardial relaxation, was also improved by reduced Ca^{++} perfusate.

Recovery of high energy phosphate stores is also enhanced by reperfusion with low Ca^{++} solutions. Using the same isolated rabbit interventricular septa preparation subjected to 45 minutes of ischemia then reperfused either with 2.5 mM Ca^{++} or 0.75 mM Ca^{++} solution, Shine and Douglas (1983) found significantly enhanced recovery of ATP and phosphocreatine. The authors suggest that low Ca^{++} reperfusion may enhance ATP regeneration by sparing ATP required for intracellular sequestration of Ca^{++} or by decreasing mitochondrial calcium loading thus facilitating high energy phosphate recovery.

Ashraf et al. (1984) found that reperfusion of globally anoxic rat hearts with solutions containing Diltiazem, a blocker of the membrane slow inward Ca^{++} current, both decreased tissue Ca^{++} after 20-30 minutes of anoxia and decreased the extent of ultrastructural injury, particularly, injury to mitochondria.

The diagram below schematizes some of the ways in which elevated intracellular calcium introduced at the time of reperfusion might precipitate further injury.



Schematic representation of the direct consequences of loss of Ca^{++} homeostasis during postischemic reperfusion.

Nayler and Elz, 1986.

Cell Swelling and the "No-Reflow" Phenomenon

Cells which are ischemic have a rise in tissue osmolality which can be accounted for by conversion of glycogen to lactate and by hydrolysis of ATP (Tranum-Jansen et al., 1981). Thus, when reperfusion with normotonic fluid occurs, intracellular accumulation of water occurs resulting in cell swelling. When canine myocardium is exposed to 40 minutes of ischemia, then reperfused with arterial blood, marked increases in tissue water, sodium, calcium and chloride with losses of potassium, indicating loss of cell volume regulation. By contrast after 15 minutes of ischemia, there are only slight increases in water and sodium. While 40 minutes of ischemia resulted in myocyte swelling and other changes of irreversible injury on reperfusion, perfusion staining of this tissue showed the vasculature to be intact. On the other hand, after 90 minutes of ischemia, perfusion staining failed to show perfusion to the infarct center, the "no reflow" phenomenon; - thus, despite restoration of blood flow in large vessels, tissue reperfusion did not occur. Examination of tissue revealed severe capillary damage, tightly packed red cells and extravasated red cells indicative of capillary disruption (Kloner et al., 1974). An additional cause of the no-reflow phenomenon is thought to be capillary plugging by leukocytes introduced during the reperfusion process (Engler et al., 1983).

Effect of Oxygen Free Radicals

Activated oxygen species or oxygen free radicals generated during the reperfusion of ischemic tissue have been the focus of much recent attention as potential mediators of reperfusion injury. Oxygen free radicals are formed when molecular oxygen accept less than four electrons and thereby form highly reactive oxygen metabolites.

$$O_2 + \bar{e} \longrightarrow O_2^{\cdot-} \text{ (superoxide)}$$

$$O_2 + 2\bar{e} + 2H^+ \longrightarrow H_2O_2 \text{ (hydrogen peroxide)}$$

$$O_2^{\cdot-} + H_2O_2 + H^+ \longrightarrow O_2 + H_2O + {\cdot}OH \text{ (hydroxyl radical)}$$

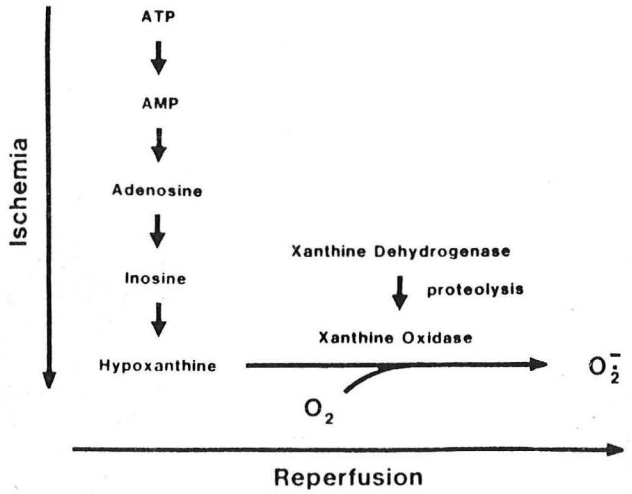
$$O_2 + 4\bar{e} + 4H^+ \longrightarrow 2\,H_2O \text{ (water)}$$

Formation of reduced O_2 species.

Grisham and McCord, 1986

Under normal circumstances, production of oxygen free radicals involves less than 5% of oxygen utilized by mammalian cells (Graham and McCord, page 1). There are endogenous enzymes, namely superoxide dismutase, catalase and glutathione peroxidase which scavenge these radicals thereby maintaining extremely low concentrations of these injurious products.

Several events during myocardial ischemia and reperfusion set the stage for free radical generation (Hammond and Hess, 1985; Werns et al., 1986). During ischemia, ATP is metabolized to hypoxanthine and the enzyme xanthine dehydrogenase is converted to xanthine oxidase. Upon reintroduction of oxygen at reperfusion, cells with increased hypoxanthine and increased xanthine oxidase liberate superoxide anion.



Proposed mechanism for O_2 radical production in ischemic tissue.

Grisham and McCord, 1986

In the presence of iron containing compounds superoxide anion and hydrogen peroxide will yield hydroxyl radical via the Haber Weiss reaction.

$$O_2^-\cdot + Fe^{3+} \rightarrow O_2 + Fe^{2+} \qquad (1)$$

$$H_2O_2 + Fe^{2+} \rightarrow HO\cdot + OH^- + Fe^{3+} \qquad (2)$$

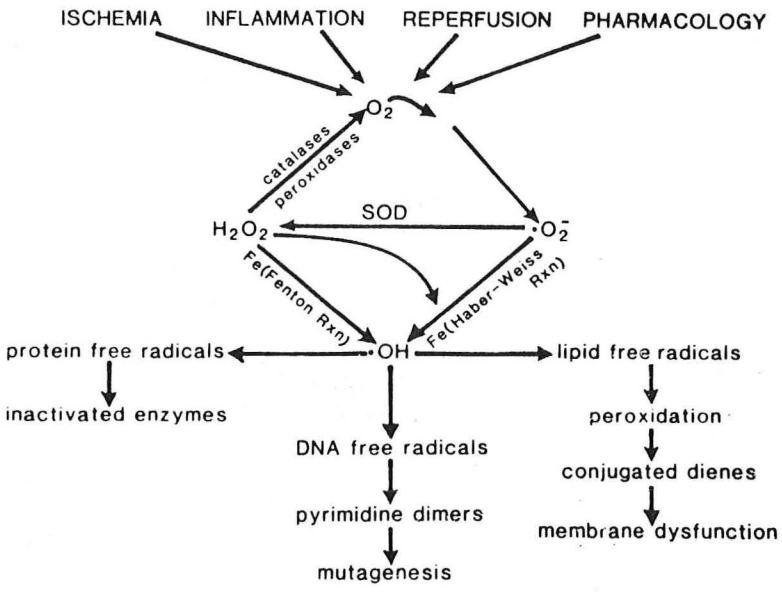
$$\text{Sum: } O_2^-\cdot + H_2O_2 \rightarrow HO\cdot + OH^- + O_2 \qquad (3)$$

Grisham and McCord, 1986

Hydroxyl radical is the most reactive and most injurious of the free radicals generated. Of note, there is no scavenging enzyme system for this radical (Hammond and Hess, 1985).

Other intracellular sources of free radicals are mitochondria, arachidonic acid metabolic intermediates resulting from Ca^{++} activated phospholipase degradation of cell membranes, and activated neutrophils accumulating at the site of ischemic cell injury (Hammond and Hess, 1985; Werns et al., 1985; Werns et al., 1986; Crisham and McCord, 1986).

A postulated schema of the widespread cell damage caused by activated oxygen species is shown in this diagram.



The proposed cellular and subcellular effects of the oxygen free radical system as a result of ischemia or reperfusion. These entities result in the univalent reduction of molecular oxygen to the superoxide anion ($\cdot O_2$). The superoxide anion can then be dismutated to hydrogen peroxide by the superoxide dismutase enzymes (SOD, MnSOD and CuZnSOD) and then either by a Fenton reaction (Rxn) or a Haber-Weiss reaction produce the hydroxyl radical, which is a potent oxidizing species. The hydroxyl radical in turn can produce protein free radicals, DNA free radicals and lipid free radicals.

Hammond and Hess, 1985.

The evidence that free radicals may play a role in myocardial reperfusion injury are based on studies measuring infarct size, ATP stores or contractile function in ischemic reperfused myocardium treated with free radical scavengers or with inhibitors of xanthine oxidase. While agreement is not uniform, there are a number of well designed studies supporting a beneficial effect of free radical scavengers. Thus, Jolly et al., (1984), Werns et al. (1985) and Ambrosio et al. (1986) all found significant reductions in infarct size with infusion of superoxide dismutase. Of importance, the study design of Ambrosio et al. (1986) began the infusion at the moment of reperfusion simulating a clinical scenario. Benefit when the intervention occurred at the time of reperfusion is supportive of the concept of an injurious effect of reperfusion separate from the effects of the prior ischemic period. Myers et al. (1985), Burton (1985), Przyklenk and Kloner (1986), and Myers et al. (1986) found significant functional recovery when superoxide dismutase was added to reperfusate. Of interest, the study of Burton (1985) used isolated buffer perfused rabbit hearts, thus showing that free radical formation occurred at sites in the myocardium rather than requiring activated leukocytes. Finally, Ambrosio et al. (1987) found improved recovery of phosphocreatine stores and contractile function when superoxide dismutase was added at the time of reperfusion.

Likewise, treatment with allopurinol, a xanthine oxidase inhibitor, is somewhat controversial. Reimer and Jennings (1985) found no effect on infarct size after treatment with allopurinol while Werns et al. (1985) found a beneficial effect. A difference in study design which may account for the difference in results is that Werns at al. (1985) pretreated their animals with allopurinol 18 hours prior to induction of ischemia allowing adequate time for metabolism of allopurinol to its active metabolite, alloxanthine.

Leukocytes as Mediators of Reperfusion Injury

Another potential mediator of cell injury are activated leukocytes drawn to a site of tissue injury. Recent interest has focused on the contribution of leukocytes to infarct size as reperfusion enhances delivery of leukocytes to ischemic myocardium (Engler et al., 1983; Rossen et al., 1985; Engler et al., 1986a; Engler et al., 1986b). Leukocytes are thought to have two adverse effects - early on after ischemia, they have been shown to cause capillary plugging with the result that coronary vascular resistance increases and coronary artery blood flow to the microvasculature is further decreased (Engler et al., 1983). Despite subsequent restoration of blood flow to the large arteries and arterioles, microvascular blood flow may be limited by capillary plugging, i.e. the "no reflow" phenomenon (Engler et al., 1983). A second injurious effect of such entrapped leukocytes is the release of superoxide anion, proteolytic enzymes and platelet activating factor, all of which may add to the injury of ischemia thus increasing infarct size (Bednar et al., 1985). Although leukocytes arrive at the ischemic zone by collateral flow, their influx is greatly facilitated by reperfusion. When leukocyte function is inhibited (Bednar et al., 1985) or leukocytes are depleted (Engler, 1986a) experimental infarct size is decreased and microvascular reperfusion is greatly increased.

Myocardial Hemorrhage During Reperfusion

When ischemic tissue is reperfused, hemorrhage into the area of injury occurs. There was some concern that hemorrhage would cause additional injury and thereby extend infarct size. Morphologic studies (Fishbein et al., 1980; Roberts et al., 1983), however reveal that hemorrhage occurs within the area of advanced necrosis where injury is irreversible and thus does not increase infarct size.

In conclusion, I would like to address the issue of how this laboratory derived data can be applied to improve the results of clinical thrombolysis. Two general avenues of inquiry are required. First, efforts should be made to optimize patient selection so that those most likely to benefit from reperfusion can be identified. The second approach is to select additional therapy designed to control the deleterious consequences of reperfusion.

PATIENT FACTORS

A. Residual Stenosis

Most studies reporting results of thrombolytic therapy (Andersen et al., 1983; Kennedy et al., 1983; Khaja et al., 1983; Anderson et al., 1984; Collen et al., 1984; Leiboff et al., 1984; Rentrop et al., 1984; Van de Werf et al., 1984; Holmes et al., 1985; Relman, 1985; Rentrop, 1985; Topol et al., 1985; Erbel et al., 1986; Fung et al., 1986; Gissi Study Group, 1986; ISAM Study Group, 1986; Jang et al., 1986; O'Neill et al., 1986; Serruys et al., 1986; Simoons et al., 1986) compare patients according to whether or not antegrade flow can be reestablished. Unlike animal models, patients usually have considerable atherosclerosis with a superimposed clot resulting in coronary artery occlusion. Therefore, when thrombolysis is successful, antegrade flow is generally reestablished across a severe residual stenosis. If you recall for a moment the animal lab studies on the relationship between restriction to blood flow and function, function declined with decreases in blood flow. Thus, the presence of a severe residual stenosis may limit recovery of function despite successful clot dissolution. Sheehan et al. (1985) tested this hypothesis by examining recovery of regional contractile performance in 47 patients in whom thrombolysis was successful. They found that regional recovery was significantly better in patients with a minimum stenosis diameter of 0.4 mm than in those with minimum diameters less than that figure. O'Neill et al. (1986) approached the same question somewhat differently by randomly assigning patients with acute myocardial infarction to either streptokinase therapy or to percutaneous coronary angioplasty. In this study 83% of streptokinase and 85% of PTCA patients had successful restoration of antegrade blood flow. However the degree of residual stenosis (expressed as

percent of normal vessel diameter) was significantly different between the two groups (83 $\pm$ 17% residual stenosis in the streptokinase group versus 43 $\pm$ 31% stenosis in the angioplasty group. Only 4% of angioplasty patients had a residual stenosis $\geq$ 70%, while 83% of streptokinase treated patients had such severe residual stenosis. When thallium perfusion scanning was compared in the two groups, 47% of the streptokinase treated patients had evidence of residual ischemia compared to 14% of the PTCA treated patients. Global ejection fraction as well as regional ejection fraction was significantly improved in patients undergoing PTCA. Importantly, when patients with comparable duration of ischemia in the two groups were compared, these with angioplasty had a better outcome.

These papers would suggest that in addition to the presence of antegrade flow, the quantity of that flow may very importantly determine functional recovery, a finding in agreement with laboratory data.

B. Collateral Flow

The intensity of ischemia influences the time course and extent of functional recovery. While intensity of ischemia is difficult to measure in patients, Rogers et al. (1983) approached this issue by comparing patients in two categories - a "no-flow" group with total occlusion of the infarct related artery and absent collaterals compared to a "limited-flow" group with subtotal occlusion of the artery or total occlusion of the artery with visible collaterals. The average interval to therapy was 7 hours. They found that despite equal success at reestablishing antegrade flow in the infarct related artery, improvement in global ejection fraction occurred in only those patients with limited flow via collaterals or subtotal occlusion.

Schuler et al. (1982) compared the size of thallium perfusion defects prior to thrombolytic therapy with that after thrombolytic therapy in a small group of 21 patients. The largest decrease in perfusion defect (and thus, largest increase in tissue salvage) occurred in those patients with extensive collateralization or with only subtotal occlusions.

While the above studies are conducted in small groups, they at least begin to investigate intrinsic patient factors other than ischemic duration modulating anatomic and functional recovery of myocardium after thrombolysis.

ALLEVIATION OF REPERFUSION INJURY

Therapy to control the deleterious effects of reperfusion will need to be effective when administered at the time of restoration of blood flow. Approaches suggested by the laboratory data might include agents to control calcium entry, free radical scavengers, leukocyte inhibition, and substrate to enhance high energy phosphate regeneration.

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