After the Heart Attack: Limiting the Damage

This is the sixth in a series of Research News articles examining recent developments in research on heart disease.

Although researchers have identified at least some of the steps that people would have to take in order to reduce the toll taken by heart attacks, the fact is that heart attacks claim nearly 700,000 lives every year in the United States. Seventy percent of the deaths occur outside the hospital, but coronary care units and the availability of drugs to control the potentially fatal disturbances in heart rhythms that may result from damage to cardiac muscle have helped to improve the chances for survival of those victims who actually reach the hospital. Now cardiologists think that the survival rates could be further improved by therapies that limit the amount of cardiac muscle killed as a result of blockage of the coronary arteries.

This optimism contrasts with the pessimism of previous years. Until the 1960's, it was thought that when the blood supply to a portion of the heart muscle was sharply reduced, the muscle died and there was little or nothing that anyone could do about it. However, more recent research has shown that although this might be true for part of the area affected, there is a borderline zone that may recover. Moreover, the evidence indicates that the extent of the recovery can be influenced by the treatment the patient receives. It is not only necessary to avoid harmful therapies but it appears possible to institute beneficial ones.

The basis for much of the work is the understanding that blockage of one or more of the three major coronary arteries by atherosclerotic lesions or blood clots rarely cuts off all blood flow to the affected portion of the heart. Collateral arteries, which are small vessels branching from nearby open arteries, can carry some blood to the tissue, but the flow is reduced so that the supply of oxygen is less than the demand for it. Tissue that is not receiving an adequate blood supply is called "ischemic." If the imbalance between oxygen supply and demand is high or persists for a long enough time, the ischemic tissue may die. The dead area is called an "infarct." The experiments of Robert Jennings, Keith Reimer, and James Lowe of Duke University Medical School have shown that severely ischemic tissue dies very rapidly but that the affected area also contains moderately ischemic tissue that may recover. Consequently, many of the strategies to limit infarct size aim to increase the supply of oxygen to the heart, to decrease the demand for it, or, if possible, to do both.

One of the pioneers in this research is Eugene Braunwald, working first at the University of California at San Diego (UCSD) and more recently at Harvard Medical School. According to Braunwald, John Ross of UCSD, and Peter Maroko, who is now at Harvard, the most important factors that influence the heart's demand for oxygen are tension development or degree of stretch in the heart wall, the contractility of cardiac muscle (how fast and forcefully the muscle fibers can shorten), and the heart rate; increases in all of these increase oxygen consumption.

In experiments on dogs, the investigators found that drugs, some of which are used to treat heart attack victims, may in some circumstances actually cause infarct enlargement. This is true for isoproterenol, for example. Isoproterenol increases the amount of blood pumped by the heart by increasing contractility and the heart rate. Although increasing the cardiac output might appear to be desirable for a patient whose heart is pumping poorly after a heart attack, the results of the Braunwald group indicate that there is danger of causing the size of the infarct to increase.

The encouraging result from a number of laboratories, including Braunwald's, is that certain therapies can prevent extension of the area of dead tissue. A smaller infarct means that the risk of dangerous arrhythmias and death are decreased, whereas the patient's chances of leading a more normal life with less disability are increased.

Most of the research thus far has been carried out on animals, but a few drugs, including nitroglycerin, hyaluronidase, and propranolol, have undergone preliminary trials in humans. The investigators think that the early experiments have been successful enough to warrant more extensive clinical trials.

Experiments by Stephen Epstein, Jeffrey Borer, and their colleagues at the National Heart, Lung, and Blood Institute (NHLBI) showed that administration of nitroglycerin to dogs decreased the size of infarcts produced by tying off one of the coronary arteries. The drug also lowered the susceptibility of the hearts to ventricular fibrillation, a dangerous heart arrhythmia.

The NHLBI investigators and another team of cardiologists at Johns Hopkins University Medical School have recently tested the effects of nitroglycerin on a limited number of humans with heart attacks. Both groups found that the drug improved heart function and reduced the extent of the injury caused by ischemia. The researchers used electrocardiograms (ECG's) to assess the extent of the damage. The magnitude of the deviation from normal of a certain ECG abnormality is known to correlate with the size of the ischemic area, and Braunwald and Maroko have shown that it also correlates with eventual infarct size. Reduction in the magnitude of the abnormality as observed by the investigators following nitroglycerin treatment suggests that the drug has acted to reduce the extent of the damage.

The results of the two groups were similar in that both showed that nitroglycerin alone improved the condition of patients with failing hearts. (Not all heart attack victims suffer heart failure.) However, the NHLBI investigators found that the effects of nitroglycerin were unpredictable in patients without heart failure unless they were also given a drug to counteract the drop in blood pressure and the rise in heart rate it elicited. Although the drug potentiated the effects of nitroglycerin in these patients, it diminished the effects of nitroglycerin in patients with heart failure. On the other hand, Bertram Pitt of the Johns Hopkins University Medical School said that their study indicated that a drug to raise blood pressure reversed the beneficial effects of nitroglycerin in both groups of patients.

The reason for the difference is not clear, although the two teams of investigators administered the nitroglycerin by different routes. The NHLBI investigators gave the patients nitroglycerin tablets to be held under the tongue, whereas the Johns Hopkins workers injected the drug intravenously. Pitt says that with the latter route, it is easier to control the dosage to avoid large drops in blood pressure or increases in heart rate. Nevertheless, Epstein says that these effects

make it necessary to exercise extreme caution in the administration of nitroglycerin to patients without heart failure no matter what route of administration is used.

For many years clinicians feared that attempts to treat any acute heart attack patient with nitroglycerin would do more harm than good, even though the drug was an established and successful treatment for angina pectoris, a condition in which an individual experiences chest pain during exertion because the coronary arteries have become too narrow to supply adequate blood to the heart in times of increased oxygen demand. Because nitroglycerin increases the heart rate it could increase oxygen consumption by cardiac muscle. And lowering the blood pressure could decrease the flow of blood through the coronary arteries, if not all of them are blocked. These effects are just the opposite of those desired.

Moreover, the hypotensive action of nitroglycerin might put the patient in greater jeopardy from "pump failure," which occurs if the damaged heart cannot pump effectively enough to maintain blood pressure. As the blood pressure drops the heart muscle receives still less blood, pumps even less efficiently, and the vicious cycle may result in death.

Prediction of the effects of blood pressure alterations can be complicated because a decrease would also lower the work done by the heart and thus its demand for oxygen. For example, in an early experiment, Burton Sobel and his colleagues at the Washington University School of Medicine showed that using the drug trimethaphan to lower the blood pressure of heart attack victims who have hypertension reduced their short-term mortality rate and the size of their infarcts in comparison to those of controls. Since a particular therapy may produce opposing effects on the cardiovascular system, the net effect often depends on the patient's condition. Careful evaluation is needed to determine whether the balance between the effects will be beneficial or harmful.

Although nitroglycerin appears to be a therapy with a beneficial balance, the mechanism by which it limits infarct size is uncertain. Epstein and his associates have evidence that the drug increases the flow of blood through the collateral arteries to the ischemic area. He also thinks, as does the Johns Hopkins group, that nitroglycerin decreases the work of the heart.

Investigators have known for sometime that the drug decreases the amount of blood returned to the heart by dilating the veins so that the vessels contain more blood. This would decrease the pressure inside the left ventricle (the large chamber of the heart that pumps blood to all parts of the body except the lungs, which receive their blood from the right ventricle). The Johns Hopkins investigators have observed such a decrease after nitroglycerin treatment. The lower pressure would reduce the tension on the ventricular walls and the oxygen demand of the heart.

Braunwald and Maroko are encouraged by the results of their clinical trials with the enzyme hyaluronidase. Although the number of treated and control patients was small—a total of approximately 100 in two studies—Braunwald says that analysis of the ECG's of the control patients and those treated with the enzyme indicate that the agent is effective in reducing the amount of heart muscle that is destroyed following a heart attack. The researchers had previously shown that it would do so in dogs with occluded coronary arteries.

Mode of Action of Hyaluronidase

The mode of action of hyaluronidase is still uncertain. The enzyme breaks down hyaluronic acid, which serves as the cement that holds connective tissue together. Braunwald hypothesizes that this may facilitate the diffusion of nutrients through the extracellular space to cells in the area of reduced blood flow. Experiments with dogs showed that the amount of hyaluronic acid between cells in the ischemic area decreases after treatment of the animals with the enzyme. Other animal experiments indicated that the enzyme diminishes the damage to the small blood vessels of the heart following a coronary occlusion. Both these effects could help to maintain the oxygen supply to cardiac muscle.

According to Braunwald, the use of hyaluronidase has several advantages. The toxicity of the enzyme is slight, and it only rarely elicits an allergic response. In contrast to nitroglycerin and other drugs being tested for limiting infarct size, hyaluronidase does not have potentially hazardous effects on heart activity or blood pressure. And its use does not require special equipment and patient monitoring that might only be available in major clinical centers.

Propranolol is a third drug currently being tested in humans for its effects on infarct size. Several investigators, including those in the groups of Braunwald and Jennings, observed that this drug decreased infarct size in dogs. Early results from a number of laboratories indicate that propranolol may do the same in humans.

The drug has several effects that could contribute to this action. It blocks the activity of norepinephrine, a neurotransmitter that increases heart rate and cardiac contractility and stimulates a variety of biochemical reactions; propranolol thus decreases oxygen consumption by the heart but it is not clear whether its effects on cell death are due to the decrease or to blockage of the direct effects of norepinephrine on certain reactions in the ischemic cells. The evidence concerning whether or not the drug also increases blood flow to ischemic heart muscle is equivocal. Pitt and his coworkers found that it did, whereas Jennings found that it did not.

Drugs are not the only means of influencing the heart's oxygen consumption and supply. Mechanical counterpulsation techniques are being explored in several laboratories. In one such technique, called intra-aortic balloon counterpulsation, a balloon is inserted into the aorta, the main artery leading out of the heart. The balloon is inflated when the heart is at rest between contractions. This is the time when blood flow through the coronary arteries is strongest. Inflating the balloon raises the pressure in the arteries and, as a result, increases the heart's blood supply. The balloon is deflated just before contraction begins in order to decrease the pressure against which the heart contracts and thus make it easier for the heart to pump blood. Similar results can be achieved with external devices that gently compress the leg muscles and major blood vessels between heart beats and release the compression during contraction.

Investigators still need to learn a lot about the application of strategies to limit infarct size. The question of timing—how soon after a heart attack must a treatment be started and how long must it be continued in order to be effective—is an important one which has not yet been answered, although most cardiologists think that a treatment has a greater chance of success if begun early.

According to Jennings, the time that elapses before irreversible damage occurs is inversely related to the degree of ischemia suffered by the tissue. He has found that the reduction of blood flow produced by tying off one of the coronary arteries of the dog hearts is not uniform throughout the affected area. Part of the area is severely ischemic, which Jennings defines as having blood flow reduced by 85 percent. This portion undergoes irreversible damage in 20 to 60 minutes, and the chances of saving it are slim. On the other hand, mildly ischemic areas (50 to 60 percent reduction of

blood flow) are unlikely to suffer any irreversible injury. This leaves the moderately ischemic region (70 to 85 percent reduction of blood flow) as the target of salvage attempts. The onset of irreversible damage in this region, which in the dog constitutes about 30 percent of the ischemic tissue, occurs between 1 and 3 hours after the reduction in flow. Although few of the investigators were able to initiate therapy to minimize infarct size this quickly, they still obtained encouraging results. This is fortunate for heart attack victims, many of whom delay for several hours after the onset of symptoms before seeking help.

When cells are receiving a supply of blood inadequate for their needs, they undergo a variety of biochemical and structural changes. One of the major goals of investigators is the identification of the one or ones that actually cause cell death. They think that this knowledge may help them to design better therapeutic strategies for limiting the damage.

One change that occurs very early is a shift from aerobic (oxygen-requiring) to anaerobic (not requiring oxygen) processes for production of the cell's energy. Another early change is a drop in the *p*H of the ischemic cells. This occurs partly because lactic acid is the final product of the anaerobic breakdown of glucose, a major energy source for cells, and partly because the cells cannot get rid of their waste products, including the lactic acid and carbon dioxide, when the blood flow has been sharply reduced. Several investigators have shown that the *p*H drop is more severe when blood flow to the heart is stopped than when the heart is perfused with an oxygen-free fluid. The latter can at least carry off waste products.

Anaerobic processes are much less efficient than those requiring oxygen; they produce only enough energy to keep the cells alive, but not enough to support the contraction of the heart muscle cells. If a large proportion of the muscle cannot contract, the heart may go into failure. However, John Williamson of the University of Pennsylvania Medical School thinks that the drop in intracellular *p*H causes a decrease in heart contraction even before the chemical energy supplied by the aerobic processes is used up. He and his colleagues have found that both the contractile activity of the heart and the intracellular *p*H drop very rapidly following restriction of blood flow through the coronary arteries of rats. The concentration of adenosine triphosphate (ATP), the chemical form in which energy for contraction is supplied, falls only slightly during this same period of time and cannot account for the decline in contractility of the muscle.

Arnold Katz, who is now at the Mt. Sinai Medical Center in New York, proposed that a low *p*H prevents binding of calcium ions by part of the contractile mechanism of muscle cells. Since the calcium binding is a prerequisite for contraction, Williamson thinks that this effect could account for a large part of the decline in the contractility of cardiac muscle that has been deprived of blood.

Williamson and his colleagues have observed that following the reduction of blood flow to heart muscle, the ischemic tissue is composed of islands of cells that are receiving essentially no oxygen surrounded by cells that are getting enough to carry on aerobic reactions. He can distinguish between the two because in the anaerobic state certain compounds that are intimately involved in cellular metabolism become reduced whereas in the aerobic state they are oxidized. The two forms emit fluorescent light of different wavelengths.

*p*H Drop Causes Arterial Constriction

Williamson says that the drop in *p*H causes an intense constriction of the small arteries in the tissue. When flow into some of the small vessels is cut off, there may be increased flow into others. Oxygen cannot diffuse very far in the tissue because cells take it up very avidly. The result will be a patchy distribution of the gas, so that a completely aerobic cell may have a completely anaerobic cell for a neighbor.

According to James Neely of the Milton S. Hershey Medical Center of Pennsylvania State University, the decreased *p*H and, especially, the buildup of lactic acid also inhibit glycolysis, the anaerobic pathway for glucose oxidation that must supply most of the cell's energy after the oxygen supply is cut off. This would tend to make a bad situation worse. It may be possible to limit infarct size by stimulating glycolysis in cardiac muscle while at the same time inhibiting those metabolic processes that require oxygen.

Alterations in the supply of nutrients to the heart muscle may be the basis for the reduction in infarct size that is sometimes seen in heart attack patients who have been infused with a solution containing glucose, insulin, and potassium ions. D. Sodi-Pollares and his colleagues at the National Heart Institute in Mexico City introduced this therapy in the early 1960's. Since then clinical trials of the regimen have produced mixed results at best. Recently, however, Charles Rackley and his colleagues at the University of Alabama Medical Center reported that infusion with the solution reduced the mortality of heart attack patients during hospitalization. The investigators used three standard diagnostic procedures to assess the severity of the infarcts of the 70 treated and the 64 control patients. Although the severity of infarcts in the two groups was similar, the mortality in the patients treated with the solution was significantly lower than that of the controls. The mortality of the former group was from 35 to 50 percent lower than that predicted on the basis of the severity of their condition.

Rackley thinks that the solution works by reducing the availability of free fatty acids to the heart. The free fatty acids are normally a major source of energy for the heart, but other investigators have shown that the substances, which are present in elevated concentrations after a heart attack, increase the risk of dangerous arrhythmias, increase oxygen consumption by the heart, and depress its ability to contract. The Alabama investigators have shown that the fatty acid concentrations are reduced in the blood of treated patients.

The therapy should also increase the availability of glucose to the heart and its breakdown by glycolysis. However, Neely found that perfusion of ischemic swine hearts with a solution containing glucose and insulin did not increase either their glycolysis or energy production, although it did increase glucose consumption and glycolysis in normal hearts. He kept concentrations of fatty acids constant in the perfusion fluids.

Cells from ischemic tissue exhibit several kinds of structural abnormalities, especially in their outer membranes and in subcellular structures such as the mitochondria and lysosomes. Jennings thinks that loss of the ability to regulate cell volume as a result of injury to the outer membrane is an early effect of ischemia that may be the primary cause of irreversible damage. He observed that samples of heart tissue that had been irreversibly damaged by 60 minutes of ischemia swelled markedly when incubated in vitro at both 0° and 37°C; the outer membranes of the cells had holes in them. Neither of these changes occurred in control tissues. In vivo, irreversibly damaged cells swell explosively when their blood supply is reestablished, and these cells also have membrane defects similar to those of the incubated cells.

Jennings thinks that the mitochondrial changes that he and other investigators have observed may be a consequence of the defects in the cellular membrane. For example, the mitochondria of irreversibly damaged heart cells accumulate

massive quantities of calcium ions; increased movement of the ions through the defective membrane could contribute to the accumulation. In any event, mitochondrial injury could seriously handicap a cell because these structures produce most of the cell's energy.

When the lysosomes incur damage, as they do during ischemia, they release a variety of enzymes that break down cell components, cause inflammation, and further contribute to cellular injury in the affected region of the heart. Investigators have shown that corticosteroids, steroid hormones that suppress inflammation, can stabilize lysosomal and cellular membranes. Braunwald and Maroko found that the hormones have a beneficial effect on infarct size in dogs. However, the results of trials on humans have been inconsistent.

Sobel and his colleagues found that one of these steroids (methylprednisolone) actually increased infarct size and the frequency of dangerous arrhythmias. On the other hand, John Morrison of the North Shore University Hospital in Manhassett, New York, observed that the steroid decreased infarct size in some patients. The reason for this inconsistency is uncertain. Both investigators waited approximately the same time before beginning therapy, and both used a technique devised by the Sobel group to assay for infarct size.

Measuring infarct size in humans continues to be a problem for the investigators engaged in this work. In animal experiments they can directly measure the infarcts in the control and treated animals. When working with humans, the investigators must use noninvasive techniques that will enable them to determine infarct size. In addition, they must show that the infarct is smaller with therapy than it would have been without. Several noninvasive techniques now being developed (*Science*, 3 December) do not yet have the resolution or sensitivity to do this job. Consequently, most groups have used the special electrocardiographic methods developed by Braunwald and Maroko or the enzymatic one devised by Sobel and his colleagues.

The latter method depends on the fact that irreversibly damaged heart cells release an enzyme called creatine phosphokinase into the bloodstream. According to Sobel, an increase in the concentration of the enzyme in blood indicates that a heart attack has occurred and the size of the increase is correlated with the size of the infarct.

The first techniques for measuring creatine phosphokinase activity suffered from a lack of both sensitivity and specificity. Skeletal muscle and other tissues contain structural variants of creatine phosphokinase which catalyze the same reaction as the cardiac enzyme. These variants may be found in blood. The Washington University group has recently developed a radioimmunoassay specific for the heart variant; the new assay is far more sensitive than the previous method, which measured total enzyme activity. (The brain enzyme variant shares a common structure with the one from heart but the brain enzyme does not escape into the bloodstream.) Sobel thinks that the sensitivity of the radioimmunoassay may permit diagnosis of heart attacks earlier than was possible with the older method. Early diagnosis is one of the goals of cardiologists who want to limit infarct size.

—JEAN L. MARX

Homogeneous Catalysis (I): Transition Metal Clusters

Homogeneous catalysis is making an increasingly respectable showing in the real worlds of the chemical and petroleum industries. Although there are now about 20 major industrial processes that use homogeneous catalysts (see box), there are two major obstacles to even wider use of soluble transition metal complexes as catalysts: Such complexes apparently do not catalyze many reactions of the type that are currently of great interest, and the complexes are difficult to separate from the products at the end of the reaction.

The first problem is being attacked by synthesizing complexes that contain three or more atoms of the same metal (polynuclear complexes or metal clusters). To overcome the second, researchers are immobilizing mononuclear complexes on polymer or ceramic supports. At least one process in which metal clusters are used is in an advanced stage of development. (The status of immobilized homogeneous catalysts will be the subject of a second article.) But, for the most part, this attempt to broaden the range of applicability of homogeneous catalysts is still in the laboratory stage.

Homogeneous catalysts are discrete molecules, most often containing a single transition metal atom, in the gas phase or dissolved in solution. Surrounding the metal atom, attached by coordination bonds, are several chemical groups or ligands. A ligand can be an entity as simple as a hydrogen atom, but more often it is a carbon-containing group, such as the carbonyl (CO) or triphenylphosphine $[(C_6H_5)_3P]$ groups. For this reason, the catalyst molecules are also known as organometallic complexes. The reactions that they catalyze occur with both the reactants and the catalyst in the same phase. In the case of reactions in solution, the products may remain in solution or may be evolved as a gas.

The vast majority of industrial processes in which catalysts are used rely, however, on solid metal surfaces to catalyze the reaction of gaseous or liquid reactants. Not only do these heterogeneous catalysts enhance the rates of a wide variety of reactions, but, because the metal is in the form of large solid pieces or small particles imbedded in a fixed, porous support material, chemical engineers have little difficulty in devising ways to separate the catalyst from the products in commercial-scale plants.

Heterogeneous catalysts are often not highly selective and thus can be wasteful of reagents. Possibly because the surface of a metal particle exhibits several different crystal orientations, each having a somewhat different catalytic activity for the several reactions possible with a given set of reactants, the catalyst may simultaneously catalyze each of these reactions with a high probability. The loss of a large fraction of reagents in the form of unwanted products is an economic penalty, especially in an age of increasing costs of raw materials.

One reason for considering the use of homogeneous catalysts, therefore, is that, by properly selecting the ligands and the metal atom they surround to have specific electronic and steric properties, chemists can make highly selective catalysts that preferentially promote only one reaction, and thus conserve raw materials. Moreover, in homogeneous catalysts all of the expensive catalyst metal atoms are active, whereas in heterogeneous catalysts most metal atoms are buried beneath the surface of the catalyst and do not contribute to the activity.