For the heart to work as an organ, cells have to communicate. They do this via gap junctions — the seven-layered structures running vertically through the middle of this electron micrograph. On the right the heavy dots represent cross sections through myosin molecules (25 nanometers across), and the small dots are cross sections through actin filaments. Interaction between these two causes the muscle to contract. The large sausage-like structures are mitochondria, which furnish the energy for this contraction.

Cell Biology of Heart Disease

Transplants and mechanical hearts are not the only route to solving the problem of heart disease. Research on cellular mechanisms is adding to scientists' understanding of how the heart works, why it sometimes fails, and what might save heart attack victims.

by Jean-Paul Revel

CARDIOVASCULAR DISEASE has been around for a long time; one and a half million people in the U.S. have heart attacks each year. It's something that faces all of us, but it is also a disease that we can do something about.

Heart disease can appear as a slow failure (as in congestive heart disease) that makes itself felt over long periods of time, with your heart eventually just giving out. Or it can be sudden, with very little warning — one, two, three, and you are gone.

If you have a heart attack today, you have an 80 percent chance of surviving. That still leaves 350,000 people each year who don't survive. About 75 percent of people suffering heart attacks are lucky enough to be admitted to the hospital very quickly, and of those, 80 percent survive. Of the 25 percent who don't get to a hospital right away, 95 percent will die.

But I don't want to speak about what happens in hospitals. I'm a biologist, not a physician. As expressed well in a recent review (Atlas and Laragh), "There is good reason for excitement in the potential for rolling back one of the most common causes of death, but no less exciting is the process itself, in which the elaborate, elegantly logical pathways of human physiology are pieced together." We now have the beginnings of biological answers to the question of how a heart attack happens.

The heart pumps blood through a network of blood vessels 60,000 miles long. Of course
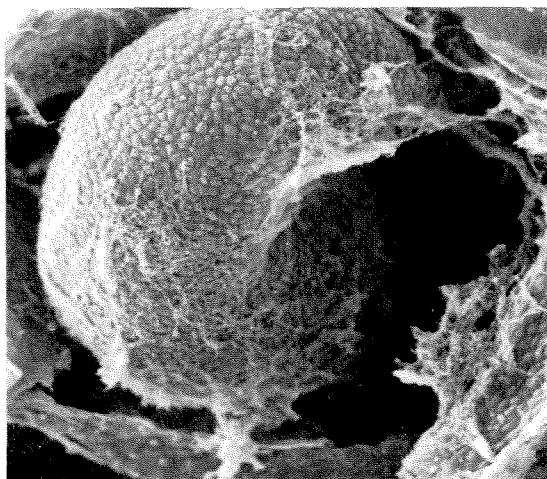
Although the adult heart is a complex organ, it starts out as a very simple tube. Shown here is the heart of a chick embryo observed under a scanning electron microscope.

Shown in diagram (top) is a single heart muscle cell with membrane, nucleus, and cytoplasm filled with contractile elements and support "machinery." Below that are some human heart cells seen through a light microscope. At this magnification only the nuclei and the "ends" of the cells, or intercalated discs, are distinguishable. In cross section (bottom) each heart muscle cell appears as roughly circular. The small crescent-shaped, dark objects between the cells are capillaries.

this isn't linear — it's a branching network — but still it's an enormous length of blood vessel that you end up having to push blood through. And the blood supply is essential. After five seconds without a blood supply to the brain you become unconscious, and by about nine minutes the damage to the brain is completely irreversible. Nine minutes is all you have from the time you fall down from a heart attack to get to someplace where oxygen can be resupplied to your blood.

The heart has four chambers, or pumps — the right and left atria and the right and left ventricles. The blood that has already circulated around the body and is depleted of oxygen enters the right atrium, where it is pushed through a valve into the right ventricle, filling it up. When full, the ventricle contracts strongly and pushes the blood with great force out into the lungs, where it is reoxygenated. Then it comes back into the left atrium and is pushed into the left ventricle, which contracts and pumps it into the aorta and around the rest of the body again.

The heart is able to work as a pump because each one of the heart muscle cells is contractile and they can all be directed to contract in concert. If we mince a heart up into individual cells, put the cells in a dish, and grow them in culture as if they were bacteria, each cell will beat by itself. Left alone the cells beat at different rates, but they will beat synchronously if allowed to make contact with each other. This is where my own interest in the heart started, because my research concerns the structures, called gap junctions, that are part of the contact area. Gap junctions are clusters of small channels through the cell membrane of two contacting cells, through which information in the form of a flow of ions can be exchanged to synchronize the two heart cells.

Although each cell is linked to every other cell and synchronized by gap junctions, there has to be something to set the pace — like a band leader. This role is played by a natural pacemaker (artificial pacemakers perform the same function). The primary one is called the sinoatrial node because it sits at the sinus, the meeting place where the blood vessels come into the right atrium. It sends out the impulse that imparts the beat to the right and left atria. Eventually that impulse reaches the atrioventricular node situated between the atria and the ventricles. The cells in the first node beat the rhythm for the atrium, and after a short delay the atrioventricular node takes up the beat, sending its impulse down the conducting system that then spreads over the ventricle. The whole thing is arranged in such a way that the beat occurs in a nice, even fashion so that the whole heart contracts in just the right way to expel the blood.

The pacemaker establishes an intrinsic rhythm to the heart, but the heartbeat is also controlled by nerves. When you get excited, your heart starts beating very hard and pushes more blood out; that's the sympathetic nerve system taking over. The parasympathetic nerve system inhibits the heartbeats. But this nerve control is not actually essential for hearts to function. When you transplant a heart, the nerves are severed and they don't grow back — yet the heart still beats.

Normally the heart beats 72 times per
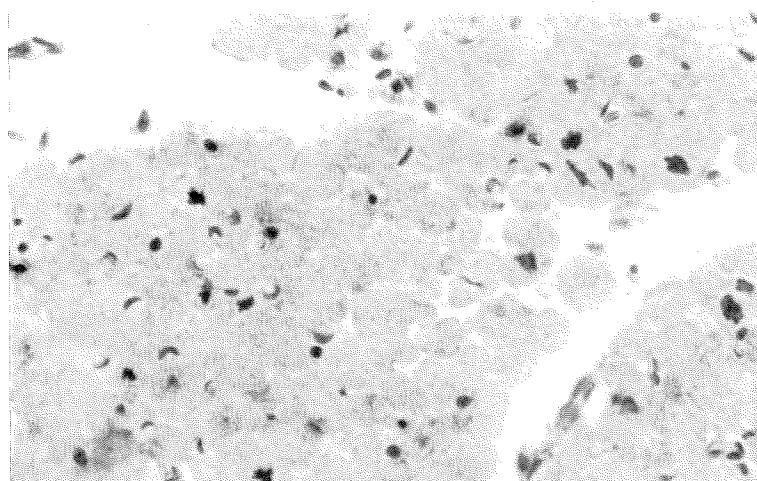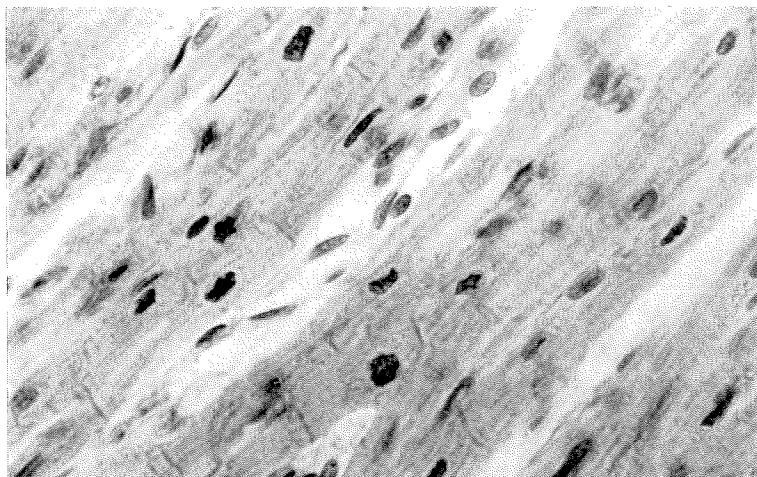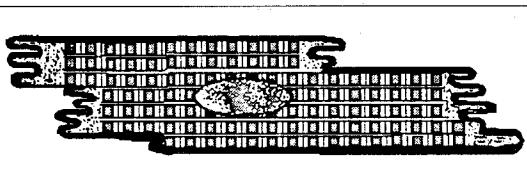
minute, but sometimes it can go too fast —
called tachycardia — or too slow — bradycar-
dia. Much worse than these is arrhythmia, in
which the heart suddenly accelerates and then
slows down, doesn't beat continuously, or
sometimes has extra beats between regular
beats. These are all disease conditions. Once
in a while it's all right to skip a beat, but if it
happens too often, you have a real problem
that has to be dealt with.

But perhaps the most dangerous thing that
can happen to the heart occurs when different
groups of cells start to contract on their own
instead of responding to the pacemaker.
Instead of contracting as a whole to push the
blood out, there are now islands of cells con-
tracting independently of each other, resulting
in a fluttering and ineffectual contraction of
parts of the heart muscle, but no contraction
of the heart as a whole. So blood doesn't get
pushed out of the aorta, it doesn't go into the
carotid artery that carries it to the brain, and
you have five seconds before you pass out
and only nine minutes before you die (or at
least sustain irreversible damage). This
fluttering is called fibrillation and is the major
cause of death from heart attacks.

The problem can be fixed by a defibrilla-
tor, a machine that passes a large amount of
current through the chest wall to try to get all
the cells to "reset." If you do it at the right
time, the pacemaker will say to the cells,
"OK, all together now," and they'll start beat-
ing synchronously again. Since defibrillators
would enable some of the 95 percent of heart
attack victims who die on the street to get to
the hospital in time, one thing that would
help is to have more defibrillators around.
But using a defibrillator without knowing
what you're doing would be like putting your
hand in an electric socket. Some of the newer
instruments being designed can sense by
measuring currents when the right time to
deliver the shock occurs, so that people who
are not trained as physicians can use them.

Now, you can't talk about the overall
activity of the heart without speaking about
the heart cells themselves. A heart cell has a
membrane, as do all cells, surrounding cyto-
plasm containing a nucleus and organelles.
The cytoplasm of muscle cells is jam-packed
with special machinery. Myofibrils, for exam-
ple, are the contractile elements that allow the
cell to shorten. Mitochondria provide the
energy to power the myofibrils. Every
myofibril is surrounded by the sarcoplasmic
reticulum, a network of tubules that stores

calcium and occupies much of the rest of the
space in the cell.

Let's look at the contractile system first.
Under the light microscope the cell with its
myofibrils looks like a succession of dark and
light stripes; the light stripe is called the
I-band for isotropic (when you look at it in
the polarizing microscope), and the dark one
is called the A-band for anisotropic. The Z-
line is located in the middle of the I-band,
and repeating units called sarcomeres connect
one Z-line to the other. These are the units
of contraction. Under the electron micro-
scope you can see two sets of filaments in the
sarcomeres — dark, thick ones made of myo-
sin and light, thin ones made of actin. The
two interact with one another in order for the
heart to contract. During contraction the two
sets of filaments slide past each other. Of
course, they can't go very far (half a micron)
because there isn't much room for them to
move, and the total contraction of the heart is
due to all these sarcomeres lined up in series
one after the other. When you sum up all of
the shortened sarcomeres, hundreds of
thousands of them end to end, you have large
movements even though each one moves
only a very short distance.

In the relaxed state the filaments overlap
less, and in the contracted state they slide in.
How do they go from one state to the other?
They do it by interacting with each other. At
rest they can't interact because a set of mole-
cules, including one called troponin, sits right
on the actin filament like a chaperone and
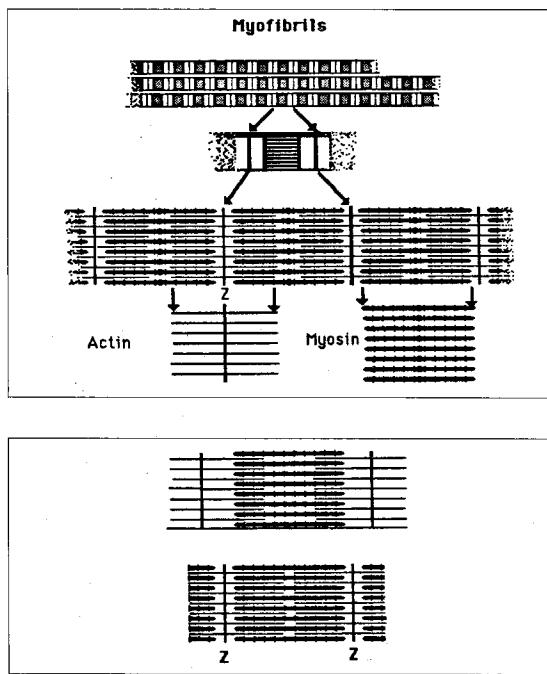
*The contractile elements, or
myofibrils, are faintly distin-
guishable as longitudinal stri-
ation in the middle picture on
the opposite page and more
readily recognizable in the
electron micrograph on the fol-
lowing page. The top diagram
shows the structure of the con-
tractile elements. Two sets of
filaments interdigitate — the
thick ones composed of the
protein myosin and the thin
ones of actin. During contrac-
tion (bottom) the actin
filaments pull in toward the
middle of a "sarcomere,"
which as a result becomes
shorter. Summing all the
shortenings causes the macro-
scopic contraction of the heart,
squeezing the blood into the
arteries.*
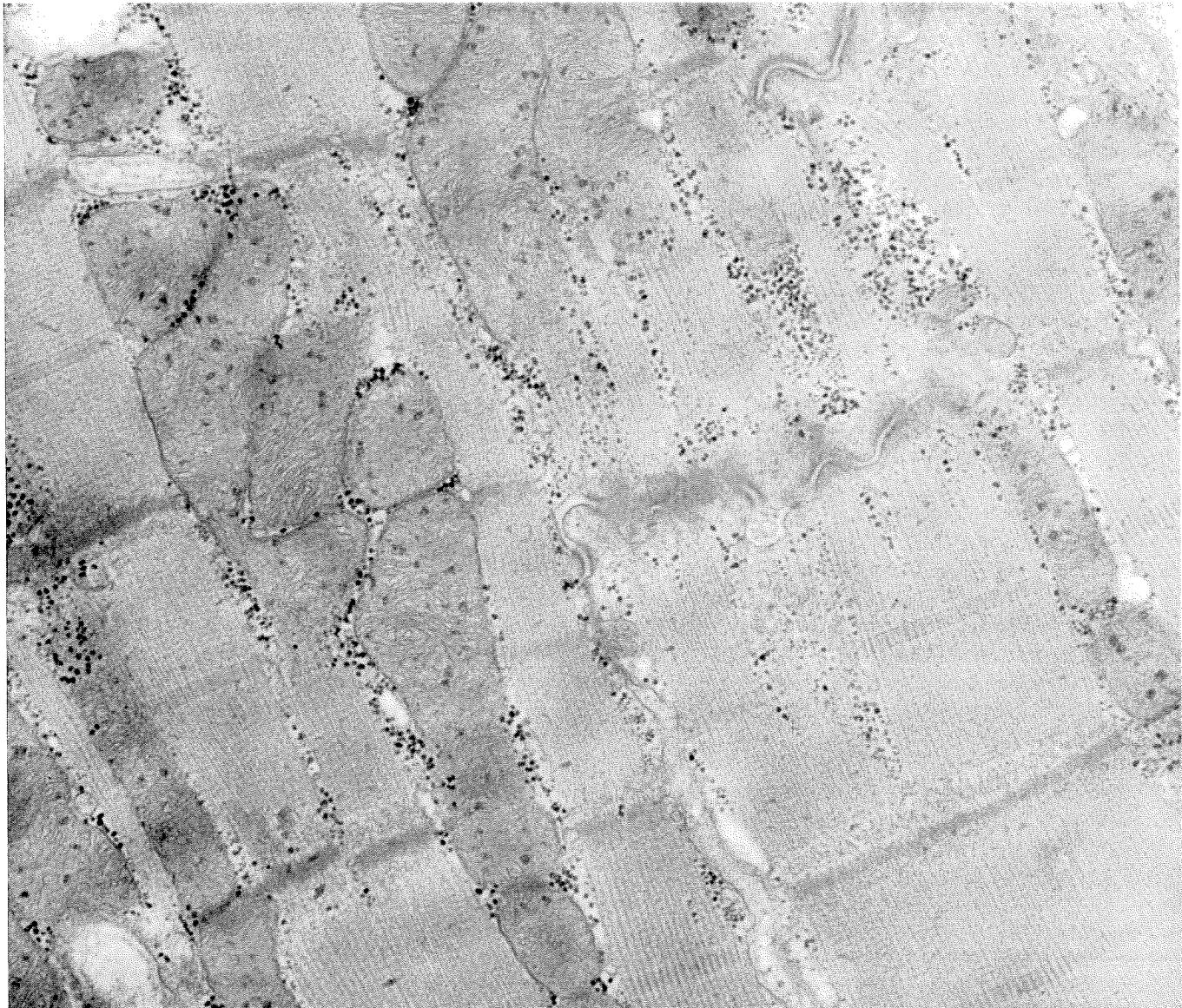
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prevents it from interacting with the myosin. If they can't interact, they can't contract. Like all chaperones, this one has faults. In many novels chaperones love chocolates, and if you bring a box of chocolates, the chaperone will sit in the corner and eat them and forget about what's happening in the back room. In the case of heart muscle, it isn't chocolate — it's calcium. This is where the calcium-storage organelle comes in, which has released its calcium as part of the coupling between excitation and contraction. The chaperone grabs the calcium, to which it binds strongly, and, as it does so, it exposes the reactive sites on the actin. The actin and myosin can interact, and everything is very good. But the calcium-storage organelle also represents a mechanism for removing the calcium. And when the calcium concentration in the cytoplasm drops low enough, the

chaperone has to give up its calcium. It can now go back and inhibit actin-myosin interactions again, and the heart relaxes. All this happens in each cell every time the heart beats — 72 times per minute.

Now let's look at the ionic events that control contraction of the heart muscle cell. Across the membrane of this cell there is a potential, -90 mV inside, which means that there are more negative charges inside than there are outside. This is an equilibrium potential due to the distribution of sodium ions that are floating around. It's a little like the situation on the Mexican border. The sodium ions, for example, hang around on the outside of the border, unable to cross because the membrane is impermeable to them. They try to get across and can't, so they bounce back. The potassium ions on the inside of the cell, however, are able to

move back and forth freely.

When the muscle decides to contract, it does so by means of an action potential — a mechanism caused by leakiness of the membrane to sodium ions. In the resting state they couldn't get across the membrane border, but during passage of the impulse to contract in the form of an action potential, the cell says, "OK guys, we'll let you in." So the sodium ions can come in. This brings in positive ions from the outside, which makes the inside less negative. The membrane potential drops; the cell is depolarizing, with the major consequence that when the cell becomes sufficiently depolarized, a gate suddenly opens that allows some calcium ions to come in too. These calcium ions then react with the calcium-storing organelle, causing it to release all its calcium and diverting the "chaperone." Now actin can react with myosin, and the muscle contracts.

After a relatively long time (all of this takes milliseconds), the inside of the cell has become even more positive, so potassium ions can leak out in a grand fashion, and they do. An outward leak of positive ions is the same as adding negative charges to the inside, so the membrane starts to repolarize — it becomes more negative inside, and pretty soon calcium can't come in any more because its gate closes again. That shuts off the whole system. All the calcium released in the cytoplasm will be picked up by the calcium-loving organelle, the myosin and actin stop interacting, and the muscle can relax. We end up with both sodium and potassium on the inside, and a membrane molecule (Na,K ATPase) that acts like the border police rounds up all of the sodium ions and kicks them out. Each time it kicks out two sodiums, it can take in three potassiums. Eventually all the sodium will be out and most of the potassium will be in, and we are ready to start the whole story all over again. This all works just great, but there is one hitch. Processes such as pumping the calcium into the storage organelles and rounding up the sodium ions require energy, which we get in the form of a molecule called adenosine triphosphate (ATP), manufactured by the mitochrondria. The mitochrondria take in phosphate, fuel in the form of sugar derivatives, and oxygen, and make CO_2 and ATP out of them. Where do they get the oxygen? From the blood supply.

All cells make ATP and use it to carry out any function in the body that requires energy,

so they all need oxygen from the blood. Since the cells of most organs use only about half of the oxygen that passes by them, those organs can increase their demand for oxygen and still find plenty available. But the heart uses 80 percent of the oxygen that goes by it in each pass. There's almost no marginal reserve. This brings us to the importance of the coronary artery system — the blood supply to the heart. Although the heart pumps blood, it doesn't use the blood that it has inside of it for its own needs. The coronaries service the heart muscle's needs. When there's a blockage in one of the branches of
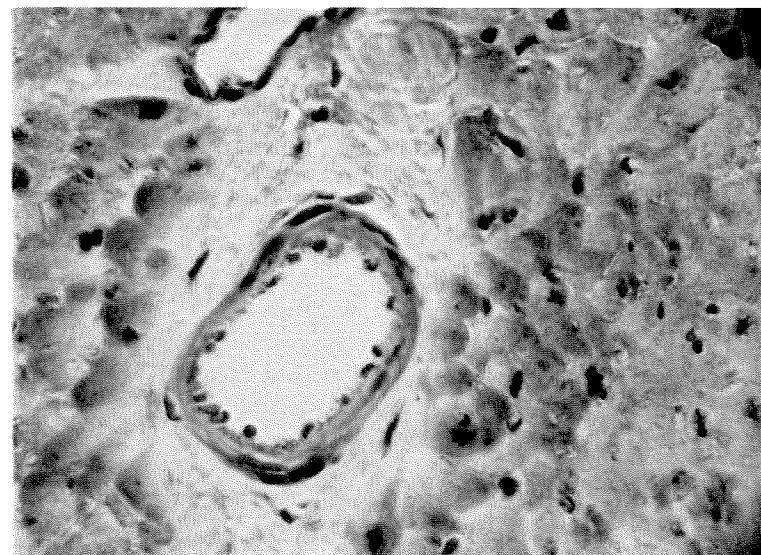
the coronary arteries, a small portion of the heart muscle doesn't get the oxygen it should get.

One of the scenarios for the consequences of such a blockage goes like this: Within seconds the calcium starts to leak out because the mitochondria stop working. We have no more oxygen; it can't get in because the blood flow has stopped. If the mitochondria have no more oxygen, they can't make ATP. Even then, the cell has still one way to make a little bit of it — about 4 percent of the total ATP can be made by a mechanism that doesn't require oxygen, but this produces lactic acid. The acid leaks out of the cell, and since it is a negatively charged compound, it has to take a positively charged compound with it. Since potassium is the most abundant positive ion inside the cell, you start losing potassium from the inside. The overall effect of this and other changes is that the membrane potential of affected cells drops from -90 mV to -60 mV during the early stages of a heart attack.

As the membrane partially depolarizes,

A small artery in a human heart shows circularly arranged smooth muscle cells and dot-like nuclei of the endothelial cells, which form the inner ply of the vessel. Occlusion of such blood vessels leads to damage of the surrounding area. If the area is large enough, this can impair functioning of the heart as a whole and lead to sudden death.

synchrony is impaired. Gap junctions close, cell-to-cell communication is disrupted, and the whole system starts to fall apart. What is especially devastating is that even though only one particular area of muscle has been badly affected by a lack of blood and has a membrane potential of -60 mV, the rest of the muscle is perfectly normal with a potential of -90 mV. But when regions of the heart muscle have different potentials, electrical currents flow between them. Such currents can reenter the muscle anywhere and start a contraction. So cells contract by themselves and not on the well-orchestrated orders from the pacemakers. This starts the process we know as fibrillation. The heart muscle stops working as a pump, and the body collapses.

Let's assume that we do the only apparently reasonable thing we can do — get oxygen to the heart so it will work again. One obvious way is to circumvent the blood stoppage. If it is due to a blood clot, we can try to dissolve the clot as quickly as possible. So we inject enzymes that very specifically dissolve fibrin, the molecule that holds blood cells together in a clot, and circulation gets reestablished. Unfortunately, as part of a continuing cascade of events, compounds accumulate in the cell which can generate peroxides when oxygen becomes available again. The peroxides that form can damage the cell and the rest of the machinery. The physician tries to reestablish circulation to the cells as fast as he can. If he doesn't, the patient may die; if he does, he'll kill the cells, which explode from the effect of all the peroxide. So he's damned if he doesn't and damned if he does. One exciting possibility being explored to prevent this is to break down hydrogen peroxide with the enzyme superoxide dismutase and thus reduce the amount of cell damage.

But instead of just trying to save something after the fact — opening up a clogged blood vessel — we ought to be trying to prevent it from occurring in the first place. So we have to figure out what causes the clog. First something goes wrong with a branch of the coronary artery. Arteries have an inner lining — the endothelium — and beneath that lies a band of elastic tissue, which gives them resilience. Surrounding this are some smooth muscle cells, which can change the diameter of the vessel and thus control the amount of flow. Smooth muscle cells are usually well behaved. They just do their job and nothing else. Normally they don't multi-ply, except in the embryo, but during heart disease something very peculiar happens: The smooth muscle cells start to proliferate and make a big bump on the wall of the blood vessel, partially occluding it. Also accumulating at this bump are scavenger cells. Both smooth muscle and scavenger cells become filled with fat, forming what in the jargon is called a plaque. Blood platelets, which normally circulate through the vessel, pile up at the bump and along with other cells form the beginning of a clot.

One theory about early events leading to a heart attack goes as follows: If the wall of the blood vessel is damaged, the blood platelets stick to the site as a reaction to the injury. The platelets can penetrate through the blood vessel wall and secrete platelet-derived growth factor (PDGF), which stimulates the smooth muscle to grow. This leads to the formation of the bump on the wall. An alternate theory runs slightly differently: Muscle cells secrete their own growth factor, but the endothelium secretes heparin, which inhibits this growth factor from acting. When the endothelium is damaged, less heparin is produced, allowing the growth factor free rein to force the muscle cells to divide and make the bump.

Whichever theory is correct, neither explains how we end up with all these fat droplets deposited in the cells. The culprit is cholesterol. Cholesterol is a molecule that's insoluble in water. So how does it get carried in the blood, which is an aqueous medium? It does it by jumping onto a submarine — a particular molecule called low-density lipoprotein (LDL). The LDL carries the cholesterol, keeping it high and dry, while the whole complex is soluble.

LDL gets out of the bloodstream by binding to a receptor on the surface of liver cells. When enough receptors have LDLs attached to them, they are taken into the cell, where the receptors are separated from the LDL and sent back to the surface to continue their jobs. Cholesterol is accumulated inside liver cells as fat droplets.

This happens normally in the liver and adrenal glands, which have a lot of cholesterol. Michael Brown and Joe Goldstein, who won a Nobel Prize last year for their work, studied a disease called familial hypocholesterolemia (FH). FH is a fairly common disease in which the receptor for LDL is defective. Most of the people who have FH — one out of 500 of us — are heterozygotes; that is, they have one good
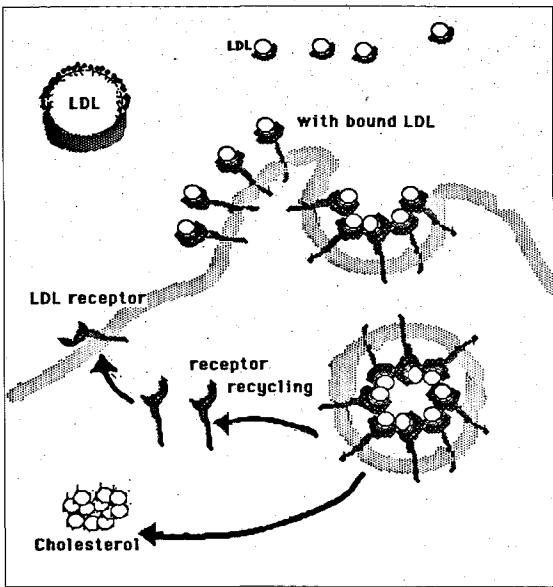
gene and one bad gene. These people have *some* good receptors for LDL but not as many as normal, and they have heart attacks at 30 to 40 years old. Those with two bad genes have almost no good receptors at all and have heart attacks in childhood. The defective LDL receptor in FH means that LDL does not get taken up by the liver, and so there's a high level of LDL in the bloodstream at all times. Just why there is deposition of cholesterol in the heart under these conditions is not understood at this point. But with high levels of LDL in the bloodstream cholesterol accumulates as plaques in the blood vessel walls, in the scavenging cells, and in the smooth muscle.

A recent theory puts the blame for this accumulation on some lipid peroxides that cause injury to the endothelium and make the smooth muscle cells, which normally would not take up much LDL, take up lots of it. Why do we care whether there is deposit of fat in the blood vessel wall? One reason is that it leads to clogging up the vessel. By reducing the diameter of the vessel, blood flow is restricted, blood platelets stick to the wall, and a clot forms. No blood at all can get through and then — heart attack!

One of the best ways to combat this situation is to prevent the whole affair from getting started. For example, we can keep cholesterol out of our diet, and this helps a little bit. It's not quite as easy as that, because if we don't take in enough cholesterol, our body makes its own anyway. So we also have to have a mechanism to control this self-manufacture. This is difficult, because you have multiple factors interacting with

each other, but progress is being made in developing drugs to counteract this. Another thing we can do is to stop smoking. Since smoking reduces the amount of oxygen the blood can carry, you don't have to have a complete obstruction of a blood vessel before the amount of oxygen arriving at your heart is reduced below a critical level.

It hasn't yet been demonstrated whether coffee is bad for you, although some studies indicate that it might be. But I've also read an article that claimed it was bad only if boiled. Alcohol, on the other hand, is good for you, because it relaxes you. Too much is no good. But none at all is bad too.

Hypertension also contributes to the kind of heart attacks I've been discussing because it puts extra stress on the heart. Narrower blood vessels make the pressure higher than it would be normally; this can often be controlled by diet and medication. Scientists have also recently discovered a hormone with the beautiful name of atrionatriuretic factor. Produced by the heart itself, it actually lowers blood pressure by working against other hormonal systems that increase the pressure.

New imaging tools — such as nuclear magnetic resonance (*E&S*, January 1986) — and other diagnostic techniques are being developed to pinpoint where the blood vessel obstruction is and how big it is. This can help physicians choose the best way of dealing with a particular case. Better transplant technology and a better understanding of the mechanism by which hearts are rejected will also save lives. Quick response by trained personnel will also help, that is, people trained in cardiopulmonary resuscitation (CPR). Most of those who enroll in CPR courses are about 20 years old, and that's very nice, but they're not the ones who are usually having heart attacks. The 50- and 60-year-olds should learn CPR — and not just wives in order to save their husbands. Women are smoking more and eating the same stuff that men do and are rapidly catching up with the men in incidence of heart attacks.

Scientists understand quite a bit about the basic biology of the heart, but there is still a lot to learn about the detailed mechanisms by which the heart does its thing. And this understanding will lead us to better ways of dealing with heart attacks. Perhaps an article such as this 10 years from now will be very different; some of the problems that we face today will have been solved. □

LDL, rich in protein, is made soluble by attaching to a protein, seen as a dark crescent in the upper left corner of the diagram. LDL receptors (left center) in the cell membrane bind to LDL. The receptors cluster (right) and are taken into the cell in a special vesicle. The fat portion accumulates in the cell, and the receptor itself is recycled back to the surface.