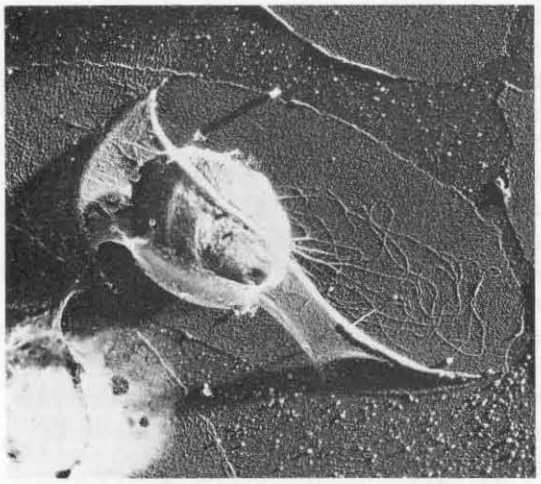
Spaced-Out Cells

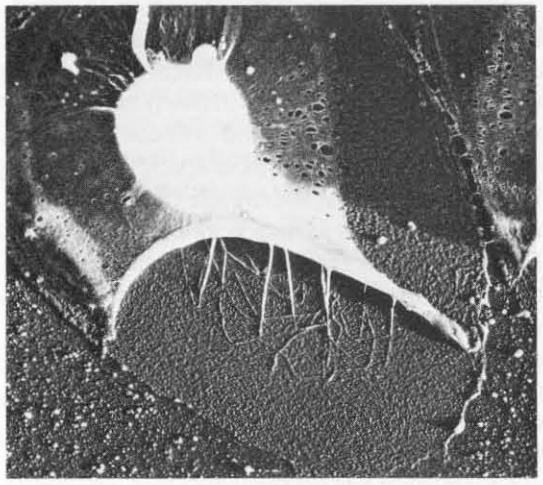
by Elias Lazarides

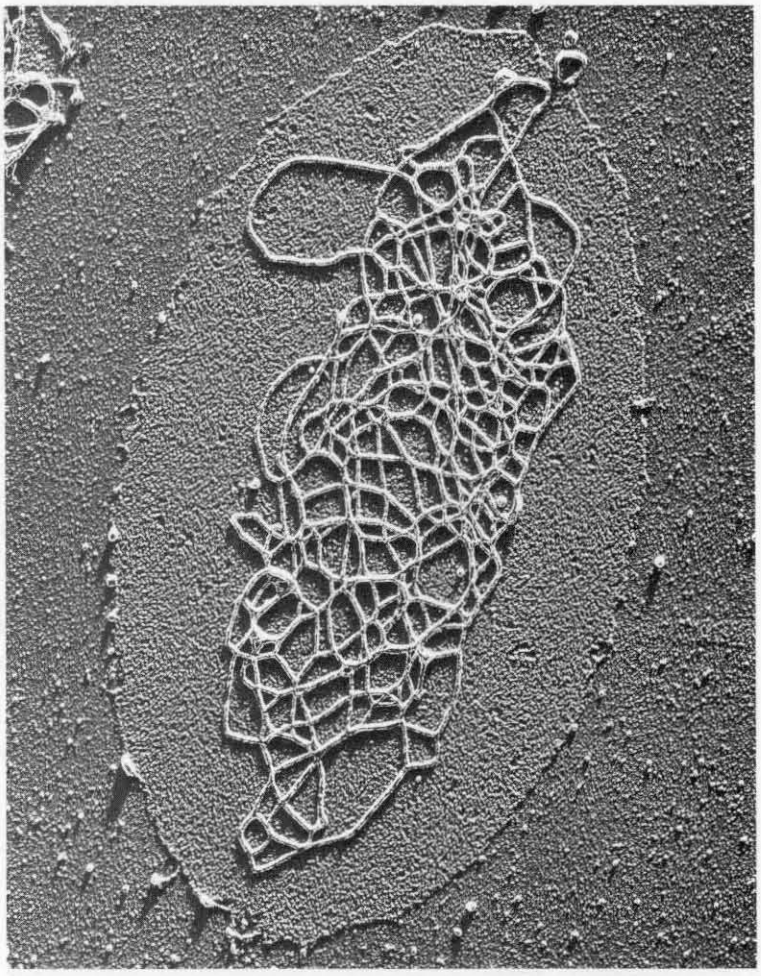
A BAG OF FLUID — that's the most common concept of a cell. But it doesn't look like that at all; a cell is a highly structured system, organized from one end to the other in three-dimensional space. The revolution in our concept of how a cell is put together has come about only in the past 10 years as the result of new technologies developed in our laboratory that have enabled us to look inside the cell and discover its complicated and integrated cytoskeleton. A small group of proteins makes up this scaffolding, which is universal among all types of cells, even though it has different functions in different cells. We have found that common principles govern what these proteins do as embryonic cells differentiate into specialized adult cells; studying these processes may bring significant insights into a number of human diseases, including muscular dystrophy and heart disease.

One way or another, every cell in our bodies is capable of being deformed. Red blood cells can serve as a simple example. There are about 5 million red blood cells per cubic millimeter in our blood, and they get squeezed thousands of times every hour through our blood vessels. Each time a red blood cell goes through a capillary, which is narrower than the normal width of the cell, the cell must be deformed from a round or oval shape to a long sausage-like structure. And after it has passed through the capillary, it resumes its former shape. The cell does this for a number of generations (about 120 days) before it peters out and is removed from circulation.

The human red blood cell has to pack millions of molecules of hemoglobin into a very small space (approximately seven microns in length and three microns in width). If the cell had nothing to keep its structure together, the osmotic pressure that the hemoglobin molecules impose on the cell would cause it to rupture eventually, especially when squeezing through a capillary. But it does have something to keep its structure together — a huge, dense (50-100 milligrams per milliliter) network of proteins, under the membrane of the red blood cell. This network is highly compacted — three to four times the viscosity of the blood — and consists of fibrous proteins (spectrin and actin) that are specifically bound under the cell membrane to control the shape of the cell and allow it to be elastic. Spectrin has been known for some time to be a component of red blood cells. We have determined its ubiquitous presence in all cells only recently, thus allowing us to envision that all cell membranes may be capable of deformability, an event that may have a common molec-







ular principle. (Postdoctoral fellows Jim Nelson and Elizabeth Repasky were particularly involved in this work.)

Elegant techniques allow us to see the shapes of these protein molecules. We can isolate a single protein from this whole network, spray it on a carbon film, coat it with platinum, and make a platinum replica of it. They are very long molecules — about 200 nanometers — and they come together end to end under the plasma membrane of the red blood cell to form a highly flexible, elastic network.

This flexible network under the membrane is not the only form of cell structure. For some reason, the chicken's red blood cell is slightly more complicated than a human cell. Unlike human red blood cells, which are cup shaped, chicken red blood cells are ovoid, football-shaped cells filled with hemoglobin; the main difference is that the chicken cell has a nucleus. The invariant position of this nucleus in the cells demonstrates another form of cell structure. If we lower the ionic strength of the medium in which the cells are isolated, the membrane will open and all the hemoglobin will spill out. But even when the cell loses all its "filling," the nucleus still stays in the center, because in addition to the band of material at the periphery of the cell, which allows the cell to keep its ovoid shape, there is another structural system superimposed on the cell's space — a system of fibers called intermediate filaments.

Research fellow Bruce Granger has devised a rather elegant technique for getting inside the cell and looking at the three-dimensional disposition of this structure. The technique involves passing sonic waves through a cell stuck onto a glass cover slip. As you increase the frequency of the sonic waves, the membrane begins to rupture, and the upper part of the membrane, which is not attached to the glass, begins to peel back. Then we can see the whole system of intermediate filaments inside the cell, spanning from the upper to the lower part of the membrane.

The intermediate filament network links the parts of the membrane together and the membrane to the nucleus like a three-dimensional gluing system. This network, also made of spectrin and actin under the plasma membrane, takes up about half of the space inside the cell, but the cell still manages to pack everything else

Rupturing the upper cell membrane with sonic waves exposes the system of protein intermediate filaments that are attached to the lower part of the membrane, seen in the electron micrograph of a red blood cell at right, above. At left, top and bottom, the network of intermediate filaments, which gives the cell structure in three-dimensional space, can be seen attaching the nucleus of the chicken red blood cell to the membrane.

in there too. In the case of the red blood cell, it packs millions of molecules of hemoglobin in between the fibers.

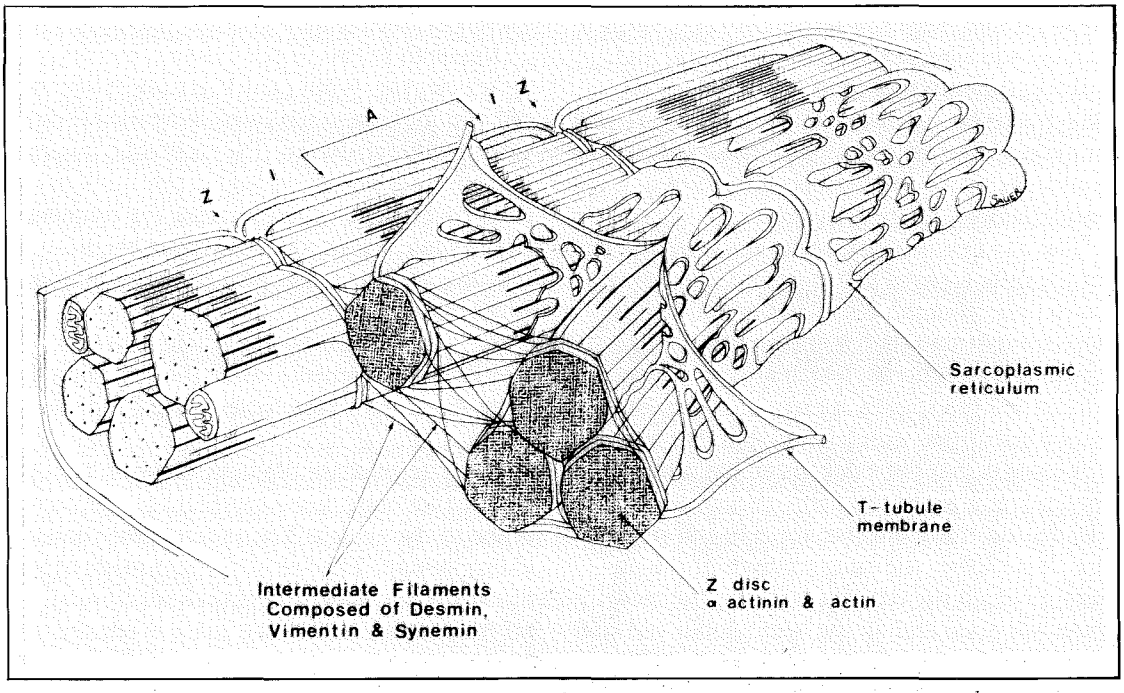
Not only are nuclei and membranes integrated by these fibers but also the mitochondria — the structures in the cell that make ATP (adenosine triphosphate), which is necessary to cell metabolism. Mitochondria have to keep an invariant position and not slosh around in the cytoplasm as cells change their shape. Again, the intermediate filaments link the outer membranes of the mitochondria all the way to the upper and lower surfaces of the cell. Every point in the cell has its own fixed place in the cell's organization.

Other cells besides red blood cells have to be elastic. For example, the lens cell of the eye has to undergo many elastic changes as the lens accommodates light. We discovered that spectrin is the protein that provides flexibility in the lens also.

Of course, not all cells deform in the same way that red blood cells and lens cells do. Muscles do something very different, but spectrin is again on the scene here performing a different function. Our muscles contract extremely rapidly, go back and forth on the order of milliseconds. Muscles manage to contract and affect our movements because they're highly striated, that is, all the contractile units, called myofibrils, are arranged along the long axis. As they contract, a great amount of tension develops along that axis. If cells did not have a way of translating this tension to lateral tension, the cell membranes would balloon out. To avoid that, imposed on the long-axis system is a lateral-axis system which apparently integrates the membrane with everything else along the lateral axis in the muscle fiber, as shown in the figure below. Intermediate filaments, with gluing struts of spectrin, keep the system of vertical and transverse axes in register. When the muscle contracts, the membrane puckers at the specific points where the spectrin struts are not attached to it, while the areas where spectrin is attached remain invariant due to their association with intermediate filaments. So, as you develop tension in one direction, you also develop tension in the other direction to keep the whole system together.

By removing the cell membrane and using detergents and different salt concentrations, we can preferentially remove different structures in the cell (in this case the myofibrils) and leave the unifying scaffold behind. We can see that fibers surround myofibrils at specific points. Looking laterally at the muscle fibers we can see how the whole system is glued together from one end of the cell to the other; it's mechanically integrated, just like the red blood cell. These fibers glue themselves at the periphery of the myofibrils to integrate the whole space and provide tension in one direction as tension is developed in another direction. And the glue is, again, a combination of intermediate filaments and spectrin.

Protein fiber structures are found performing still other functions in different types of cells, often two or three structural systems imposed on one another. In the epithelium of the



In this diagram of a muscle fiber, spectrin struts (attached to the membrane at points marked Z along the top) glue the network of intermediate filaments (web-like structure peeled out at bottom, left) to the membrane, keeping the whole system in register during contraction.

human intestine little structures called microvilli move particles of mucus up and down as we digest food. The whole system has to contract and expand, applying a great amount of tension on the columnar epithelial cells. As this takes place, the cells have to have a way of translating and linking movement from place to place. A system of filaments in one place induces movement continuously along the microvilli to move down all the particles.

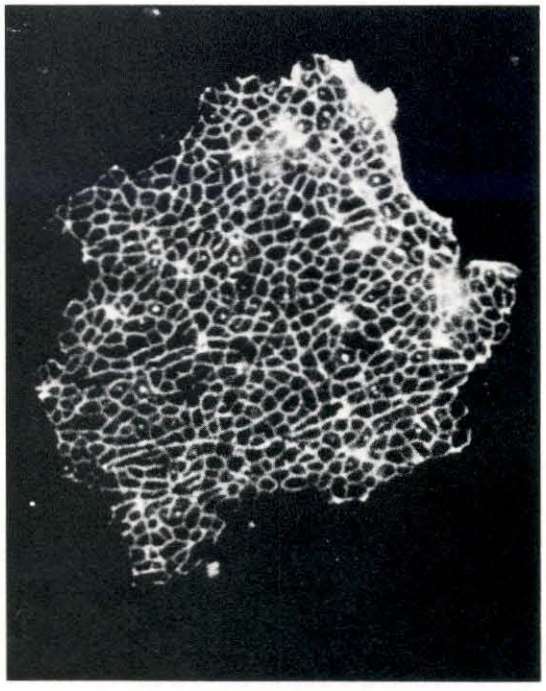
Cells also have to spend a lot of energy to keep themselves flat. When a cell is cooled, it presumably lowers its energy state by pulling all its membranes inside around the nuclear area. When it does so, it leaves behind structural material attached to specific sites of the substrate. If we revive the cell, it will expand all its membranes along tracks composed of actin filaments that provide tension to the cell.

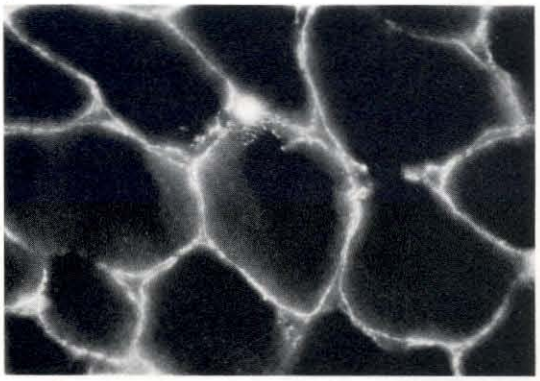
There are still other fibers of similar structure in the cell, whose function is to keep the cell in a three-dimensional shape. There's a system of geodesic (or "biodesic") structures surrounding the nuclear area and extending in a staircase-like fashion all the way to the edge of the cell.

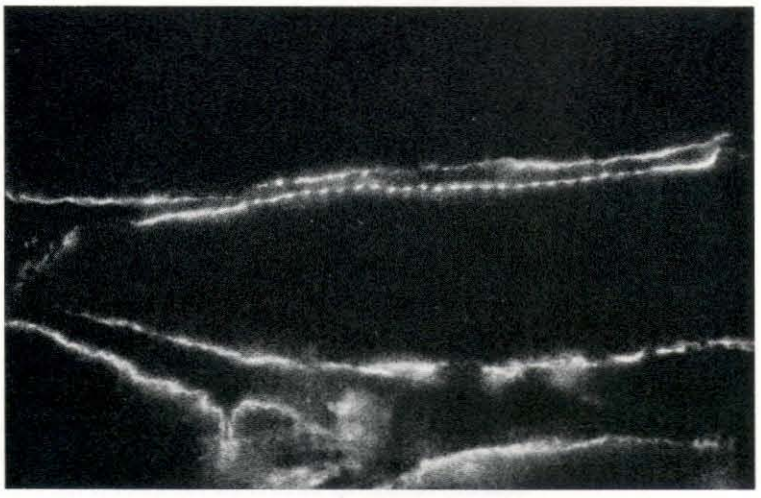
A different protein associated with actin filaments does exactly the same job of cross-linking individual filaments. This second network is found in highly motile cells that have whip-like structures. This is the same system that links the nucleus to the membrane in the red blood cell — the intermediate filaments. But in the motile cells, there is an entirely different distribution of proteins than in the static red blood cells. The amount of cross linking of the fibers has been decreased during the cell's differentiation.

From DNA cloning technologies we know that the protein molecules that enable these cells to maintain their structure, to move, and to deform their shapes are universal — they're the same in unicellular organisms of yeast and in human beings. The system diversifies itself through a complicated set of accessory proteins, which allow these same molecules to assume different functions in different cell types to produce differentiated phenotypes — red blood cells, muscle cells, lens cells, nerve cells, and so on. Also, within the same cell type, nerve cells, for example, variations of a particular protein establish segregated domains, which perform different functions during cell differentiation.

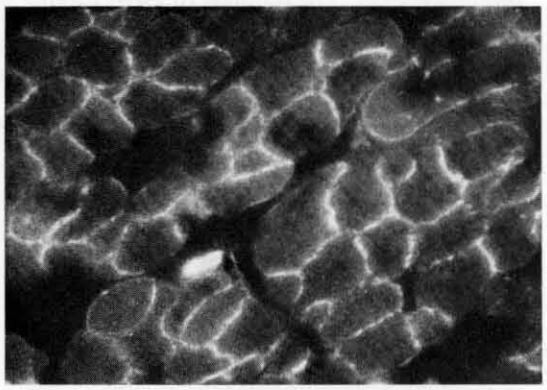
We know that each human cell contains approximately 10,000 different proteins, each of which increases in many thousand copies to bring the total of protein molecules within our cytoplasm to more than a million per cell. We have developed a technique for studying one protein at a time by making antibodies against it. We can give a protein from, say, a chicken cell, to a rabbit, and the rabbit's system will recognize it as foreign and produce antibodies







Fluorescent antibody techniques show up the intermediate filaments (far left) surrounding the myofibrils of the muscle fiber, and the spectrin under the membrane along the lateral axis (below) and in cross section (left).



In this cross section through the atrium of a chicken heart, fluorescent antibodies illuminate spectrin under the cell membranes, just as in the muscle cells on the previous page.

against it. (Sometimes we have to slightly change it chemically to trick the rabbit into recognizing the protein as foreign.) We can put a fluorescent tag (rhodamine, which is red, or fluoresceine, which is green) on this antibody, remove the membrane of the cell, put the antibodies inside, and observe with a fluorescent microscope to see where the antibodies bind. This technique has revealed that more than 50 percent of the mass of the cell is occupied by protein fibers.

We can study the biochemical composition of these fibers by making antibodies to other components, and, using different antibodies and different colors, show that different molecules co-exist in the same structure. With the fluorescent tags, we can choose a particular protein, localize it within the cell, and study its distribution during differentiation. In this way we can do molecular mapping of all the components in the cell at different stages of differentiation.

The distribution changes dramatically during cell differentiation. In the early differentiation stage of a muscle cell, as it begins to put together the contractile unit, it immediately changes the distribution of the proteins. They bind to specific structures in the cell to form the striated pattern characteristic of the adult muscle cell. We can use differentiation in tissue culture to delineate and analyze the molecular details of how the system assembles.

So far we've been looking at cells that are highly structured and specialized to do only one thing — either circulate like red blood cells, move up and down like muscle cells, or left and right like epithelial cells. There are many others — lymphocytes, macrophages, fibroblasts, which move around in our bodies during embryogenesis. Nerve cells, for example, have a very complicated system of fibers in their cytoplasm to mediate movement. They move by means of structures that look like potato chips (so we call them ruffles). Ruffles have the function of feeling the environment around the cell in three-dimensional space, allowing the cell to position itself on the substrate or onto other cells. It's interesting to watch nerve cells grow because they use these ruffles to feel other nerve cells around them — to avoid the trunks, or axons, of other nerve cells until they find the ends of the nerve cells and make contact.

With the fluorescent antibody technique we can show quite nicely what each of these structures contains. And we've discovered actin, the same molecule we found in muscles and red blood cells, doing here a very different job, which is to mediate the movement of this membrane's ruffle.

The ubiquitous spectrin is also present in nerve cells, and it appears to be the protein involved in the post-synaptic site. During differentiation we find it appearing at sites where synapses are being formed at the moment they're being formed. Actually two types of spectrin exist in the highly structured neuron, and the cell can segregate these forms of the protein in an anisotropic distribution, one form having the capacity to accumulate at specific sites on the membrane. This rearrangement of spectrin subunits into segregated domains occurs during differentiation, and we have been able to trace the stages of cell development at which one type diminishes in favor of the other or when it switches completely from one to the other.

The universal presence of such proteins as spectrin and actin have given rise to insights into how cells age, how changes in only a few proteins could affect so many parts of the human body. The changes in flexibility of skin cells, muscle cells, and so on as we age are due, at least in part, to an increase in intermediate filaments, which stiffen the cells and make them less elastic. Hypertrophy, or enlargement of the heart, is due to a vast accumulation of intermediate filaments produced to resist the tension often put on the heart muscle by athletes. The use of such drugs as steroids also increases the intermediate filaments.

We think that other forms of heart disease may also stem from anomalies in the distribution of specific proteins in heart muscle cells during cell differentiation in the embryo. And muscular dystrophic cells display a highly abnormal way of switching from one phenotype of a particular molecule to another during differentiation. We believe that this basic biomedical research into cell structure and function will inevitably result in better understanding of a large number of human afflictions and eventually lead to discoveries of new treatments. □