tus of bacteriology, University of Wisconsin; 24 March.

Max Miller, 67; professor of medicine, Case Western Reserve University; 25 March.

James H. Potter, 65; professor of mechanical engineering, Stevens Institute of Technology; 15 March.

Leo Schubert, 62; professor of chemistry, American University; 21 June.

Henry A. Schuette, 92; professor emeritus of chemistry, University of Wisconsin, Madison; 4 February.

Louis B. Slichter, 81; professor emeritus of geophysics, University of California, Los Angeles; 25 March.

Aaron S. Strauss, 38; professor of mathematics, University of Maryland, College Park; 13 April.

Edmund B. Thomas, 67; former professor of chemistry, John Carroll University; 15 March.

Otto T. Walter, 85; professor emeritus of biology, Macalester College; 6 June.

L. David Walthousen, 44; research associate for nuclear engineering, Rensselaer Polytechnic Institute; 18 May.

John L. West, 66; professor of veterinary medicine, Kansas State University; 18 April.

Hugh M. Wilson, 75; professor emeritus of radiology, Washington University School of Medicine; 21 April.

RESEARCH NEWS

Polypeptide Hormones: What Are They Doing in Cells?

Polypeptide hormones, such as insulin, prolactin, and growth hormone, are large charged molecules—not at all the kinds of molecules that can slip through a cell's membrane and enter the cell. For a number of years, researchers attributed all of the effects of these hormones to changes that occur inside the cell when the hormones bind to specific receptors on the cell surface.

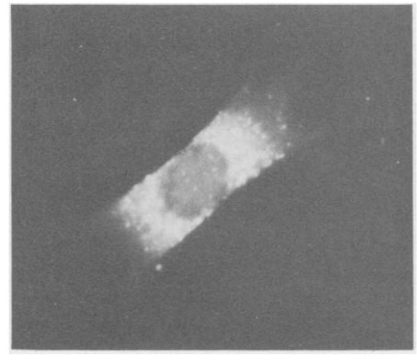
Recently, however, investigators discovered that many of these hormones do in fact enter cells, although exactly how they get in is still open to question. Seeing a whole class of new problems, researchers are jumping in to study how and why these hormones enter cells. One investigator goes so far as to say that "The internalization of polypeptide hormones is now the hottest topic in cell biology."

As so often occurs in science, the earliest reports that polypeptide hormones enter cells were largely ignored. In the 1950's, two groups of investigators published evidence that insulin enters cells, but endocrinologists persisted in believing that insulin remains on the cell surface. Twenty years later, a few scientists noticed that insulin and other polypeptide hormones may enter cells, yet even then some of these reports were greeted with skepticism.

Now opinions have changed. Once investigators accepted the fact that it was possible for polypeptide hormones to enter cells, it was easy to show that they did so. Hormones reported to enter cells include insulin, prolactin, parathyroid hormone, growth hormone, gonadotropins, and the hormone-like "growth factors" such as nerve growth factor and epidermal growth factor.

The methods used to show that hormones may enter cells can be roughly classified as morphological and biochemical. With the morphological methods, researchers look directly at cells and labeled hormones. For example, they may attach ferritin, which is an electron-dense molecule, to hormone molecules. Then they expose cells to the labeled hormone, fix the cells, and examine them with an electron microscope. With the biochemical methods, researchers look for indirect evidence that the hormones have entered cells. For example, they may show that at a certain time after cells have been exposed to hormones the hormones can no longer be removed from the cell surfaces. If the hormones are also absent from the medium, the assumption is that they have entered the cells or at least have been enfolded by the cell membrane.

Since the various morphological and biochemical methods have different advantages and different limitations, Ronald Kahn of the National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD) comments, "What is surprising is that, more or less, these different techniques seem to agree."



Fluorescently labeled α_2-macroglobulin inside mouse cells. [Source: Mark Willingham and Ira Pastan; courtesy *Cell*, vol. 13, March 1978, copyright MIT; MIT Press]

Agreement that the hormones enter cells does not extend to agreement on where the hormones go when they get inside. Researchers may be getting different results in part because their techniques are not always comparable. These differences of opinion on where the hormones go have led to differences of opinion on why the hormones enter cells.

One possible effect of polypeptide hormones entering cells may be to exert long-term effects on cellular growth and metabolism. This hypothesis is especially favored by several investigators studying insulin, a hormone whose long-term effects are poorly understood.

Insulin is the most extensively studied of the polypeptide hormones. Researchers first began to study insulin 50 years ago, yet surprisingly little is known about how it acts on cells. The hormone has short-term effects, such as changing the transport properties of the cell membrane, and long-term effects, such as changing the patterns of cell growth and protein synthesis. The short-term effects occur within minutes after insulin binds to a cell. The long-term effects occur hours later.

Many of the short-term effects of insulin seem due to the binding of insulin to its receptors on the cell surface. The long-term effects, on the other hand, have been more difficult to explain. A number of investigators speculate that the long-term effects of insulin, and possibly of other polypeptide hormones, arise when the hormones bind to specific receptors on intracellular structures.

One advocate of this hypothesis is Ira Goldfine of the Veterans Administration Hospital in San Francisco. Goldfine, A. L. Jones, and their associates report that intracellular structures, such as the nuclear membrane and the endoplasmic

0036-8075/78/0908-0895$00.75/0

reticulum (where proteins are made), have specific insulin binding sites that are immunologically distinct from the sites on the cell surface.

Goldfine and his associates also report that they can find insulin inside cells and can determine that the hormone concentrates in the nuclear membrane and the endoplasmic reticulum. To do this, they exposed radioactively labeled insulin to lymphocytes and located the insulin by electron-microscopic autoradiography.

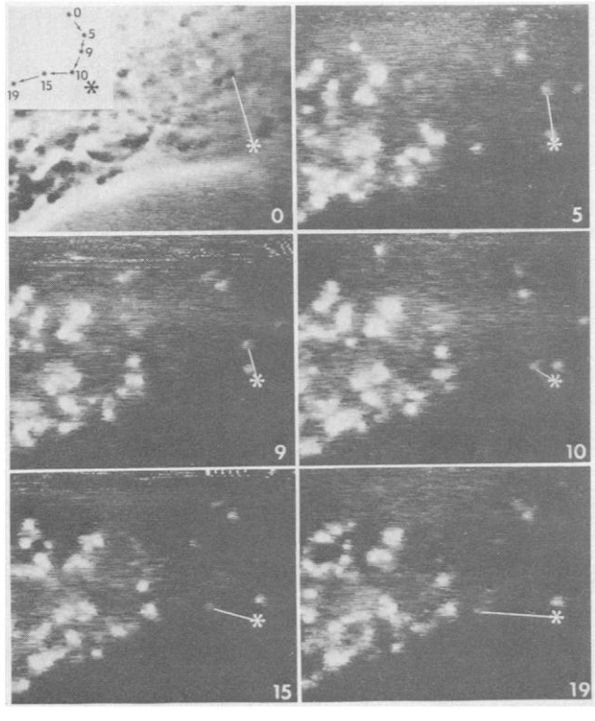
Additional support for the hypothesis that the long-term effects of hormones are due to their entry into cells comes from a group of experiments with living fibroblast cells. Yoram Schecter, Pedro Cuatrecasas, and their associates at Bur-

Image Intensification Comes to Biology

Image intensification was slow to gain popularity with biologists. It is a technique well known to astronomers, who use image-intensifying devices to amplify faint light from distant stars and galaxies. It is also well known to the military, who use it for surveillance. But biologists have relied mainly on conventional light microscopy and electron microscopy to view cells.

Since the early 1970's, only a few biologists have used image intensifiers. For example, George Reynolds of Princeton University, Lansing Taylor of Harvard University, and Werner Loewenstein of the University of Miami used this method to detect intracellular calcium by monitoring its binding to a luminescent protein. In addition, James Dvorak and his associates at the National Institute of Allergy and Infectious Diseases used image intensification to study the infection of erythrocytes by malaria merozoites.

Recently, Mark Willingham and Ira Pastan of the National Cancer Institute began using image intensification to see the movements of molecules and organelles in living cells. They use a television camera that is 1,000 to 10,000 times more sensitive than conventional television cameras. By videotaping the television images, they can continuously record molecular motions inside cells for up to 24 hours.



Saltatory motion of fluorescently labeled α_2-macroglobulin in mouse cells. A phase image was recorded at 0 time and is followed by fluorescent images. The asterisk represents an arbitrary nonmoving reference point in this field. A line is drawn in each field from this reference point to a single fluorescent vesicle. The inset (upper left) summarizes the motion of this fluorescent vesicle. [Source: Mark Willingham and Ira Pastan; courtesy *Cell*, vol. 13, March 1978, copyright MIT; MIT Press]

Willingham, Pastan, and their associates label molecules, such as polypeptide hormones and viruses, with fluorescent probes in order to see how they move in cells. They use phase-contrast microscopy to observe the motions of organelles. The advantage of image intensification is that it allows them to use so little light that they do not damage the cells.

One of the first molecules that Willingham and Pastan examined is a serum protein called α_2-macroglobulin. When they labeled it with a fluorescent probe, they saw it go inside cells and move about with a kind of motion, called saltatory motion, that has only been seen inside living cells. This is a discontinuous, dancing motion that is decidedly nonrandom. The α_2-macroglobulin molecules moved about in this way for more than 24 hours and then became associated with the lysosomes, where they were probably degraded. Interestingly, when these investigators shone enough light on the cells so that fluorescent molecules could be seen without image intensification, the saltatory motion ceased. Presumably, the more intense light damaged the cells through photooxidation of the fluorescent probes.

Joseph Schlessinger, Fred Maxfield, and Pastan also used this system to show that α_2-macroglobulin and epidermal growth factor (EGF) enter cells together and that α_2-macroglobulin and insulin enter cells together (see main story). They showed this by using two different fluorescent probes—one for α_2-macroglobulin and one for EGF or insulin. The different probes are excited by different wavelengths of light, which enables the investigators to distinguish between the two different labeled molecules and show that they enter cells together.

Schlessinger speculates that there are two reasons why biologists have not widely used image intensification. First, many were unfamiliar with the method, since there is a communications gap between biologists and physicists. The major literature on image intensification in biology has been published in biophysics journals which are not read by most cell biologists. The second reason is that biologists are very conservative about adopting new techniques.

Now that biologists are becoming aware of image intensification, it is a safe bet that many laboratories will soon be using the technique. Already several groups of investigators have indicated that they plan to buy the image-intensifying television cameras and begin experiments with this powerful method whose potential in biology has never before been realized.—G.B.K.

roughs Welcome Company at Research Triangle Park, North Carolina, prepared fluorescent derivatives of insulin and another polypeptide—epidermal growth factor (EGF). EGF, like insulin, binds to specific receptors on cell surfaces and has long-term effects on cell growth. Then Ira Pastan, Joseph Schlessinger, and their associates at the National Cancer Institute watched these labeled hormones enter living cells by means of an image-intensifying television camera (see box).

These investigators find that, when insulin and EGF enter fibroblasts, they move about in a nonrandom way as though they have specific targets in the cells. In addition, these hormones enter the cells together with a large molecule found in the serum that bathes the cells. This molecule, called α_2-macroglobulin, inhibits the action of intracellular proteases, enzymes that break down peptides. Willingham speculates that α_2-macroglobulin may protect insulin and EGF from degradation so that these two hormones can exert long-term effects on cells.

Barry Posner and John Bergeron of McGill University also have evidence that, Posner says, "raises the possibility" that insulin and other hormones may act inside cells. These investigators find receptors for insulin, prolactin, and human growth hormone on the membranous Golgi apparatus within the cells. The function of the Golgi apparatus is poorly understood. It secretes molecules from cells, but it may have other functions as well. For example, it is close to the lysosomes where molecules are degraded, and so some researchers speculate that it may deliver molecules to the lysosomes to be broken down.

Posner and Bergeron believe that the hormone receptors on the Golgi apparatus may be newly synthesized receptors on their way to the cell surface. Since the hormones do bind to these receptors, the hormone binding could also have long-term effects on the cells.

When investigators speak of hormones entering cells, they sometimes implicitly assume that it is the hormones alone that are biologically active. But most investigators believe that the hormones bring their surface receptors in with them when they enter cells. This raises the question of whether the receptors too may have a biological function in the cells.

There is some evidence that at least two polypeptide hormones take their surface receptors with them when they enter cells. Kevin Catt and his associates at the National Institute of Child Health and Human Development (NICHD) find that human chorionic gonadotropin (HCG) remains bound to its receptor when it enters ovary cells. After the interaction between the hormone and the cells was completed, these investigators broke the cells apart and separated the cell fractions by centrifugation. They found particles the size of a hormone-receptor complex in one of the cell fractions.

Another hormone that seems to bring its receptor into the cell is EGF. Stanley Cohen and his associates at Vanderbilt University find that EGF receptors disappear from the surface of cells at the same time that EGF enters the cells.

Since the hormone receptors may be entering the cells with the hormones, it is possible that the receptors rather than the hormones may be biologically active. According to this hypothesis, the hormones may be entering cells only to bring their receptors in.

There is evidence that the insulin receptor may be responsible for many of insulin's effects. Kahn and his associates find that when cells are exposed to antibodies to the insulin receptor they behave in many respects as though they were exposed to insulin. The antibody to the insulin receptor binds to the receptor but bears no chemical resemblance to insulin. Presumably when the antibody binds to the receptor, it causes the receptor to change its shape. It is this change in shape that allows the receptor to exert its effects on cells.

Kahn reports that not only can the receptor antibody cause the short-term effects of insulin but it can also cause at least one long-term effect, namely, the induction of an enzyme known as lipoprotein lipase.

Are Receptors Degraded in Cells?

Another reason why polypeptide hormones may be entering cells is to bring their receptors in to be degraded. This hypothesis is of great interest to many researchers since it provides an explanation of puzzling changes in the numbers of hormone receptors on cell surfaces.

A few years ago, it was discovered that cells behave in a seemingly peculiar way when exposed to polypeptide hormones. The more hormone a cell is exposed to, the fewer its receptors for the hormone (*Science*, 13 May 1977, p. 747). Apparently, cells get rid of surface receptors when exposed to excess hormone in order to avoid overresponding to the hormone. They gain receptors when there is little hormone around in order to make it more likely that they will pick up the hormone in their external environment.

Consistent with the idea that hormones bring their receptors into cells so that the receptors can be degraded is the discovery by a number of researchers that labeled polypeptide hormones go to the lysosomes of cells. Presumably, these labeled hormones are attached to their receptors.

Phillip Gorden and his associates at NIAMD showed that about 40 to 50 percent of the insulin that binds to liver cells enters the cells and goes preferentially to the lysosomes. Thomas Chen and his associates at Colorado State University and, independently, Catt find that HCG goes to the lysosomes in ovary cells. All three groups of researchers used electron-microscopic autoradiography to obtain their results.

Analogous to the proposal that hormones bring their receptors in to be degraded is the suggestion that hormones enter cells to be themselves degraded. Donald Steiner of the University of Chicago points out that in the past it was thought that hormones were degraded in the blood. Now, however, investigators are finding that the disappearance of insulin, at least, from the blood depends on its binding to cells. In fact, Anthony Zeleznik and Jesse Roth of NIAMD have shown that proinsulin, the precursor of insulin, and some variants of insulin stay in the blood much longer than insulin. Proinsulin and these variants have low affinities for the insulin receptors. Steiner speculates that when proinsulin remains in the blood for long periods of time it provides the body with some form of insulin between meals and during fasts.

So far, there are four possible answers to the question, What are polypeptide hormones doing in cells? Hormones enter cells to bind to intracellular receptors and exert long-term effects. They enter cells to bring in receptors so that they can act on cells. They enter cells to bring in their receptors for degradation. And they enter cells so that they themselves can be degraded. These answers are not necessarily incompatible. The polypeptide hormones have many effects on cells, and the different effects could have different causes. In addition, the different hormones may have different mechanisms of action.

A few years ago it was heresy to claim that polypeptide hormones get into cells. Now the heresy has become the gospel, and the gospel has many followers who believe it holds the key to understanding how polypeptide hormones work.

—GINA BARI KOLATA